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Кафедра микробиологии, вирусологии и иммунологии

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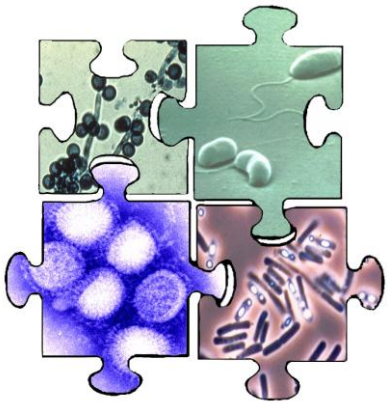
МИКРОБИОЛОГИЯ, ВИРУСОЛОГИЯ И ИММУНОЛОГИЯ

Учебно-методическое пособие
для студентов 2 и 3 курсов факультета
по подготовке специалистов для зарубежных стран медицинских вузов

MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY

Teaching workbook for 2 and 3 year students
of the Faculty on preparation of experts for foreign countries of medical higher educational institutions



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В учебно-методическом пособии представлены тезисы лекций по микробиологии, вирусологии и иммунологии, рассмотрены вопросы морфологии, физиологии и генетики микроорганизмов, приведены сведения об общих механизмах функционирования системы иммунитета и современных иммунологических методах диагностики инфекционных и неинфекционных заболеваний. Приведены сведения об этиологии, патогенезе, микробиологической диагностике и профилактике основных бактериальных и вирусных инфекционных заболеваний человека.

Может быть использовано для закрепления материала, изученного в курсе микробиологии, вирусологии, иммунологии.

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Lecture 1. MICROBIOLOGY. MORPHOLOGY OF THE BACTERIAL CELL. PHYSIOLOGY OF PROKARYOTES. CULTIVATION OF BACTERIA

Microbiology is the study of microorganisms.

Microorganisms are organisms, invisible to the unaided eye (microscopic object = microbe).

MICROBIOLOGICAL METHODS OF THE RESEACH:

1. **Bacterioscopical** (microscopical) method.
2. **Bacteriological** method (cultivation).
3. **Biological** method.
4. **Immunological** method (includes express-diagnostics, serotyping, serodiagnostics or serological method, skin testing or allergological method and methods of estimation of the immune status).
5. **Molecular-genetic method.**

OBJECTS OF THE MICROBIOLOGY:

1. **Eukaryotes** (fungi, some algae and protozoa).
2. **Prokaryotes** (blue-green algae and bacteria).
3. **Viruses, prions** and **viroides**.

OBJECTS OF THE MEDICAL MICROBIOLOGY:

1. **Bacteria** (→ bacterial infections).
2. **Viruses** (→ viral infections).
3. **Fungi** (→ mycosis).
4. **Protozoa** (→ invasions).

MODERN CLASSIFICATION OF BACTERIA

In the previous edition of *Bergy* classification (1993) all bacteria were divided into 4 divisions: *Gracilicutes* (Gram-negative bacteria with thin cell wall), *Firmicutes* (Gram-positive bacteria with thick cell wall), *Mendosicutes* (archebacteria with a defect cell wall), and *Tenericutes* (cell wall-less bacteria). According to the new edition (2001) all bacteria have been reclassified on the base of degree of the genetic relationship (16S component of ribosomal RNA). There are 3 **domains** among all the cellular forms of microbes: "**Bacteria**" (eubacteria), "**Archaea**" (archebacteria) and "**Eukarya**" (eukaryotes).

The domain "Bacteria" includes 23 types of bacteria. The majority of bacteria are combined into type (phylum) ***Proteobacteria*** which is further classified into alpha-, beta-, gamma-, delta- and epsilonproteobacteria. They generally include Gram-negative bacteria. Phylum **Firmicutes** and **Actinobacteria** include Gram-positive bacteria.

Taxons are used in bacteriology:

1. Domain ("Bacteria").
2. Phylum (type).
3. Class.

4. Order (name is ended with –ales).
5. Family (name is ended with –ceae).
6. Genus.
7. Species (basic taxon in microbiology).
8. Intraspecific variants or types (biovar/biotype, serovar/serotype, phagovar/phagotype).

Strain (isolate) is a microbial culture, isolated from the certain source (soil, water, and human) and characterized differing from others within the species. **Pure culture** is a culture of the same microbes grown on the solid nutrient medium.

Serotype is an intraspecific variant of the bacterial species characterized by the certain set of antigens.

Phagotype is an intraspecific variant of the bacterial species characterized by certain sensitivity to bacteriophages.

NOMENCLATURE OF BACTERIA:

Binomial system is used.

The first word in the name is the genus, with the first letter always capitalized. The second word is the species name, generally beginning from the small letter. For example, *Escherichia coli → E.coli, Staphylococcus aureus → S.aureus.*

METHODS OF MICROSCOPY:

1. **Electron microscopy**
2. **Light microscopy:**

- Basic light microscopy;
- Immersion microscopy (most frequently use in bacteriology);

Principle of immersion microscopy: immersion oil is placed between the glass slide and objective lens ⇨ eliminates losses of light rays getting in the objective lens.

- Dark-field microscopy;
- Phase-contrast microscopy;
- Fluorescent (luminescent) microscopy.

METHODS OF STAINING:

1. **Simple staining** (staining by methylene blue or by aqueous fuchsine);
2. **Differential (complex) staining:**

- Gram staining (revealing of the cell wall structure);
- Ziehl-Neelsen staining (revealing of the spores or Acid Fast Bacteria/AFB);
- Neisser staining (revealing of the volutin granules of *Corynebacterium diphtheriae*);
- Burry-Hines staining (revealing of the capsules);
- Zdrodovsky staining (revealing of Rickettsia);
- Romanovsky-Giemsa staining (revealing of Spirochetes and the blood parasites).

MORPHOLOGICAL FEATURES OF BACTERIA:

1. **Shape** and **size** of the bacteria.
2. **Arrangement** of the bacterial cells in a smear.

3. Structural components (organoids):

• **Obligate (basic):** nucleoid, cell wall, cytoplasmic membrane, mesosomes, ribosomes.

• **Facultative:** plasmids, inclusions, capsules, spores, flagella, fimbriae (pili).

• **Protective:** capsule and endospores.

• **Additional:** inclusions, flagella, pili (fimbriae).

4. **Tinctorial properties** (ability to be stained).

5. **Mobility** of bacteria.

SIZE OF BACTERIA:

• **Cocci** — 1 micrometer (1 mkm);

• **Rods:** very small (coccobacteria), small and average (most of the rods) and large (branch-forming and spore-forming rods);

• **Spirochetes** — thin and long bacteria;

• **Mycoplasma** — bacteria which have no constant size.

SHAPE OF BACTERIA:

1. **Cocci** (spherical bacteria).
2. **Rods** or **bacilli**.
3. **Helical** or **spiral forms** of bacteria (Vibrio, Spirilla, Spirochetes).
4. **Bacteria without the certain shape** (Mycoplasma).
5. **Bacteria with the filamentous shape** (Actinomycetes).
6. **Bacteria resemble viruses** (Rickettsia and Chlamydia).

ARRANGEMENT OF COCCI IN A SMEAR:

1. **Micrococci** (single cocci without special arrangement).
2. **Diplococci** (groups of two cells)**:**

• **pneumococci** (lanceolate or elongated diplococci);

• **gonococci, meningococci** (bean-shaped diplococci);

• **enterococci** (oval diplococci).

3. **Tetrads** (packet arranged from the number of cells, multiple to 4).
4. **Sarcina** (cubical packets of 8, 16, 32 cells).
5. **Streptococci** (chain of cells).
6. **Staphylococci** (groups of cocci resemble bunches of grapes).

ARRANGEMENT OF RODS IN A SMEAR:

1. Rods without any order — **monobacteria** (most of the rods).
2. Pairs forming rods — **diplobacteria** (Klebsiella, Corynebacteria).
3. Chains forming rods — **streptobacilli** or **streptobacteria.**
4. Rods are arranged at the angles to each other (Corynebacteria).

SPIRAL BACTERIA:

1. **Vibrio** (comma shaped curved rods).
2. **Spirilla** (rigid spiral forms with few coils).
3. **Spirochetes** (flexuous spiral forms with many coils).

CELL WALL OF BACTERIA

• General structure: the main component of the bacterial cell wall is a **peptidoglycan**, which consists of two major subunits. Glycan portion (backbone) is composed of two amino sugars, N-acetylmuramic acid and N-acetylglucosamine. The peptide portion consists of a chain of several aminoacids, which join the glycan backbones.

• **Gram staining** is a method of revealing of the cell wall structure. Steps: staining by gencian violet, treatment by iodine solution, alcohol decolorization, staining by fuchsine. Result of Gram staining: Gram-positive bacteria are violet, Gram-negative bacteria are pink.

• **Cell wall of Gram-positive bacteria:**

1. Many layers of the peptidoglycan (thick cell wall).
2. Teichoic acids and lipoteichoic acids.

Table 1 — Morphological groups of the bacteria according to the Gram state

Gram-positive group of bacteria	**Gram-negative group of bacteria**
1. Majority of cocci: ✓ Streptococci and Pneumococci ✓ Staphylococci	**1. Exclusion from cocci:** ✓ Gonococci ✓ Meningococci
2. Spore-forming rods: ✓ Bacilli ✓ Clostridia	**2. Non spore-forming rods**: ✓ Enterobacteria (*E. coli*, Salmonella, Shigella, Citrobacter, Serratia) ✓ Coccobacteria (Yersinia, Bordetella, Brucella) ✓ Others: Pseudomonas, Haemophilus, Legionella, Gardnerella
3. Rods with irregular form: Corynebacteria and Mycobacteria	**3. Spiral forms of bacteria**: Spirochetes, Spirilla, Vibrio
4. Rods with regular form: Listeria	**4. Bacteria which are resemble viruses:** Rickettsia and Chlamydia
5. Branch-forming rods: Actinomycetes	**5. Cell wall-less bacteria** (Mycoplasma) and **bacteria with defect of cell wall** (L-forms)

• **Cell wall of Gram-negative bacteria**:

1. Single layer of the peptidoglycan (thin cell wall).
2. Periplasmic space (periplasm).
3. Outer membrane (includes phospholipids, lipoproteins and the most important component is LPS or **l**ipo**p**oly**s**accharide).
4. Porins (proteins).

LPS consists of O-antigen (O-Ag), core polysaccharide and lipid A (toxic component of LPS → second name of LPS is endotoxin).

Cell wall-less forms of bacteria:

1. **L-forms** are the bacteria which lack cell wall but can grow and divide (L-transformating agent are antibiotics, lysozyme, aminoacids, physical factors; L-forms may produce chronic or persistent infection);

2. **Protoplasts** are Gram-negative bacteria which lack all cell wall (can't dividing); **Spheroplasts** are Gram-positive bacteria which lack the most of cell wall (also can't dividing).

FLAGELLA AND BACTERIAL MOTILITY

• **Flagella** are the filamentous structures attached to the cell surface that provide the swimming movement of bacteria.

• Spirochetes have periplasmic or internal flagella (axial filaments).

• Composition: protein flagellin.

• The presence or absence of flagella and their number are characteristics of different genus of bacteria.

• Cocci are immobile bacteria. Spiral bacteria are mobile. Rods can have different mobility (*E.coli* is mobile, Shigella is immobile).

Classification of bacteria (rods) according to number and localization of flagella:

1. Monotrichous (polar flagella, single).
2. Polytrichous (many flagella):
   - ▫ Amphitrichous (flagella at both poles of the bacterial cell).
   - ▫ Lophotrichous (tufts of flagella localized at the end of the cell).
   - ▫ Peritrichous (flagella are arranged around the cell).
3. Atrichous (lack of a flagella).

• Flagellum consists of several rings embedded in the cell envelope (basal body), hook-like structure and flagellar filament. The inner rings (M- and S-rings), located in the plasma membrane. The outer rings (P- and L-rings), located in the periplasm and the outer membrane respectively.

• **Detection of the flagella and bacterial motility**:

1. Flagellar staining (impregnation method).
2. Electron microscopy.

2. Motility test medium demonstrates if cells can swim in a semisolid medium (spreading type of bacterial growth).

3. "Hanging drop" method or "pressed drop" method (demonstrates the motility of the microorganisms).

FIMBRIAE (PILI)

• **Fimbriae** are short, hair-like structures on the surfaces of bacteria. Like flagella, they are composed of protein **(pilin).**

• Fimbriae are very common in Gram-negative bacteria, but occur in some Gram-positive bacteria as well.

• Function: the major factor of bacterial virulence because they allow pathogens to attach and colonize the tissues and/or to resist attack by phagocytes.

• Types of fimbriae: **F-pili** or **sex pili** (mediate conjugation) and **common pili** (almost always called **fimbriae**) are usually involved in the specific adherence.

CAPSULE

• **Capsule** is a layer outside of the cell wall.

• Composition: polysaccharide or polypeptide.

• Type of capsule: <u>microcapsule</u> (produced by the majority of bacteria) and <u>macrocapsule</u> (some bacteria).

• Capsules are most pronounced in such bacteria:

✓ Klebsiella (always form the capsules even when growing on media).

✓ Pneumococci, meningococci.

✓ Bacilli causing anthrax.

✓ Many coccobacteria.

• Functions: adherence of the bacteria and protection of the bacteria from the immune factors (phagocytosis and antibodies).

• Method of revealing is Burry-Hines staining. Steps: Indian Inc for background, then — fuchsine. Result of Burry-Hines staining: dark background, capsules are colorless, bacteria inside of capsules are pink. After other simple or complex staining the capsules are visible as colorless areas around bacteria.

SPORES OF BACTERIA

• **Endospore** is a resting form of the bacteria for defence in the unfavourable conditions of external environment.

• **Exospores** are the reproductive structures in streptomycetes.

Differences of the exospores and endospores:

1. Not resistant in unfavorable conditions of environment.
2. Forms outside of the bacterial cell.
3. One bacterial cell contains many exospores.

• Composition of endospores: DNA, cytoplasm with some enzymes, plasma membrane, spore wall (normal peptidoglycan), cortex (thick layer of peptidoglycan) and keratin spore coat (exosporium).

• Resistance of endospores is provided by:

1. Practical absence of unbound water.
2. Increased calcium concentration.
3. Presence of dipicolinic acid.
4. Especial composition of a protein.
5. Especial composition of the cortex.

• Conditions for sporulation: external environment and artificial media.

• Spore-forming bacteria are **Bacilli** (with central, small oval endospores) and **Clostridia** (with central/subterminal/terminal, big, spherical endospores).

• Method of revealing is Zhiel-Neelsen staining. Steps: Zhiel fuchsine and heating of a smear; decolorization by acid, staining by methylene blue. Result: spores are pink, vegetative cells are blue.

ATYPICAL BACTERIA

SPIROCHETES

• Taxonomy: three genera — **Treponema** (→ endemic treponematoses and epidemic syphilis), **Borrelia** (→ borrelioses: epidemic and endemic relapsing fevers, Lyme borreliosis) and **Leptospira (**→ leptospirosis**)**.

• Morphology: Gram-negative spiral forms of bacteria, motile. Treponema has 8–12 regular coils, Borrelia — 3–8 irregular coils, Leptospira — many primary coils, few secondary coils, shape like C or S and hooks like ends. They are facultative intracellular parasites.

• Ultrustructure: outer membrane contains many lipids; there are several **axial filaments** (endoflagella) in a periplasm for motility.

• Methods of revealing: study of mobility with using dark field microscopy (“hanging drop” method); **Romanovsky-Giemsa staining** (Treponema — pink; Leptospira — red; Borrelia — blue).

ACTINOMYCETES

• Taxonomy: three genera — **Actinomyces** (→ actinomycosis), **Streptomyces** and **Nocardia** (→ nocardiosis).

• Morphology: Gram-positive brunch-forming rods, nonmotile, can produce exospores. Actinomyces can form microcolonies — yellow sulfur granules (**druses**) in the pus during the disease (center of a druse is Gram-positive, peripheral part is Gram-negative).

• Methods of revealing: Gram staining.

MYCOPLASMA

• Taxonomy: two genera — **Mycoplasma** (→ mycoplasmosis) and **Ureaplasma** (→ ureaplasmosis).

• Morphology: smallest free-living bacteria, **"membrane parasites"**. Gram-negative, cell wall-less polymorphic bacteria, nonmotile. They have *three-layered plasma membrane* which includes sterols (unlike other bacteria).

• Methods of revealing: phase-contrast and electron microscopy.

RICKETTSIA

• Taxonomy: genera **Rickettsia** (→ rickettsioses: epidemic and endemic typhus, spotted fever, scrub typhus, etc.); **Bartonella** (→ trench fever); **Coxiella** (→ Q-fever).

• Morphology: Gram-negative smallest polymorphic bacteria (rods or coccobacteria). They are **obligate intracellular parasites** (resemble viruses), can't produce NAD and glycolytic enzymes.

• The Rickettsia frequently has a close relationship with arthropod vectors (ticks, lice) that may transmit the organism to mammalian hosts. Rickettsia must be grown in the laboratory by *cultivation in chicken embryo, cell culture* or *laboratory animals* and they have not been grown in artificial medium.

• Methods of revealing: Romanovsky-Giemsa staining (dark blue rods on the light blue background of the host cell); Zdradovsky staining (pink rods on the blue background of the host cell).

CHLAMYDIA

• Taxonomy: genera **Chlamydia** (→ trachoma, chlamydiosis, "inclusion" conjunctivitis) and **Chlamydophila** (→ pneumonia, bronchitis, psittacosis).

• Morphology: Gram-negative smallest polymorphic bacteria (cocci). They are **obligate intracellular parasites** (resemble viruses), they can't produce ATP (chlamydia are "**energy parasites**").

• Chlamydia can produce **intracellular inclusion bodies** (microcolonies) near the nucleus inside of the host cells.

• Chlamydia is unique bacteria — they have **cycle of development** (life or growth cycle). There are revealed infectious elementary body (EB) which develops into a noninfectious reticulate body (RB) within a cytoplasmic vacuole in the infected cell. The RB divides by binary fission to form particles which, after synthesis of the outer cell wall, develop into new infectious EB progeny. The yield of chlamydial EB is maximal 36 to 50 hours after infection.

• Methods of cultivation and revealing of Chlamydia are the same as for Rickettsia. Only *cytological method* can be used for revealing of the inclusions. Also can be used detection of the inclusion antigens.

PHYSIOLOGY OF MICROORGANISMS:

- Types of microbes nutrition.
- Types of microbes respiration.
- Cultivation of bacteria.
- Biochemical activity of bacteria.
- Variability of bacteria.
- Formation of toxins and other factors of pathogenicity.
- Sensitivity to antibiotics, bacteriophages, bacteriocins.
- Others biological properties.

Bacterial metabolism is a combination of the physical and chemical processes providing living activity of a microbial cell. Metabolism consists of two main directions: **anabolism** (biosynthesis of polymeric compounds — proteins, NA, polysaccharides — from monomers) and **catabolism** (biodisintegration of complex polymeric compounds).

NUTRITIONAL REQUIREMENTS

1. **Carbon** (is required for all bacteria growth):
a) autotrophs (use carbon dioxide as source of carbon);
b) heterotrophs (use organic compounds as source of carbon).
2. **Inorganic ions**:
a) halotolerant bacteria (resistant to salts);
b) halophilic bacteria (require for growth high concentration of salts).
3. **Growth factors** (vitamins, purines, pyrimidines, and aminoacids):
a) prototrophs (wild strains which don't require growth factors);
b) auxotrophs (mutant strains which need growth factors).
4. **Electron donors**:
a) litotrophs (use reduced inorganic compounds as source of energy);
b) organotrophs (use reduced organic compounds).
5. **Electron acceptors** play essential role in respiration and fermentation:
a) oxygen is terminal electron acceptor in aerobic respiration;
b) pyruvate, lactate and other organic compounds are products of the terminal electron acceptance in fermentation.

OXYGEN REQUIREMENTS

1. **Obligate aerobes** (require oxygen).
2. **Obligate anaerobes** (oxygen inhibits their growth).
3. **Facultative anaerobes** (able use both electron acceptors — oxygen and organic compound, can have aerobic respiration and fermentation).
4. **Microaerophilic bacteria** (grow best under decreased oxygen tension);
5. **Capnophilic bacteria** (grow best under increased carbon dioxide tension).
6. **Aerotolerant bacteria** (can survive, but do not grow for a shot period of time in the presence of oxygen).

Tolerance to oxygen is related to presence of the enzymes:

✓ **Catalase** (breakdown of hydrogen peroxide).

✓ **Superoxide dismutase** (neutralize the toxic oxygen radicals).

ENERGY METABOLISM

1. **Fermentative metabolism** (use the organic compounds as both electron donors and electron acceptors, includes Glycolytic pathway, Entner-Duodoroff pathway and Pentose phosphate shunt).

2. **Respiratory metabolism** (use aerobic respiration).

3. **Autotrophic metabolism** (use photosynthesis and anaerobic respiration).

PLASTIC METABOLISM

1. Aminoacids biosynthesis.
2. Nucleotide biosynthesis.
3. Macromolecules biosynthesis (RNA, DNA, proteins).
4. Peptidoglycan biosynthesis.

GROWTH

Factors affecting growth of bacteria in the laboratory:

1. Media (nutritive media)

Classification of media:

a) *On consistency*:

- Liquid media (MPB — meat-peptone broth).
- Semisolid media (semisolid MPA).
- Solid media (MPA — meat-peptone agar).

b) *On composition*:

- Simple media (MPB and MPA).
- Complex media.
- Synthetic (or semisynthetic) media.
- Natural media.

c) *On destination*:

- **Fundamental (basic) media** — MPA and MPB (for cultivation of the unp retentious bacteria); also *blood agar (serum agar)*.
- **Selective media** (for pure culture getting only of the certain bacterial species; for example, use of *salt agar* for Staphylococci).
- **Differential-diagnostic media** (DDM) or indicator media → for getting pure cultures and simultaneous differentiation of the different species by their biochemical activity; for example, Endo agar is used for all Enterobacteria, but *Escherichia coli* gives lactose-fermenting pink colonies while *Shigella dysenteriae* gives lactose-nonfermenting colorless colonies.
- **Enrichment media** (for accumulation of the certain groups of the bacteria in the pathological material before inoculation).
- **Transport media.**

2. Temperature

(a) Methophilic bacteria (grow best at 20–40 °C, optimal t = 37 °C).

(b) Thermophilic bacteria (grow best at 50–60 °C).

(c) Psychroplilic bacteria (grow best at 10–30 °C).

During typical bacterial growth bacterial cells divide by a binary fission and their mass and number increase in the exponential manner.

Bacterial growth curves consist of several stages:

1. Lag phase (intense physiologic adjustment).
2. Log phase (maximal rate of cell division).
3. Stationary phase (balance between cell growth and cell death, sporulation).
4. Decline or death phase (bacterial cells undergo lysis).

Microbiological (bacteriological) method of investigation (stages):

1. First day of investigation:

- Preliminary microscopy of the pathological material.
- Inoculation of the pathological material on an agar plate ► to get isolated colonies (obtaining of *the pure culture).*

2. Second day of investigation:

- Study of the cultural characteristics of the colonies: shape, size, surface (R- and S-colonies), pigmentation, opacity, elevation, edges, etc.
- Re-inoculation of the suspected colonies on a slant agar ► for accumulation of pure culture.

3. Third day of investigation:

IDENTIFICATION of pure culture includes:

- Check of purity of a pure culture (morphological properties).
- Study of the biochemical properties of bacteria (revealing of the proteolytic and saccharolytic enzymes, catalase test, oxidase test, etc.).
- Estimation of a bacterial antigenic structure (detection of serotypes).
- Study of the bacterial sensitivity to bacteriophages — viruses of bacteria (detection of the phagotypes).
- Revealing of the pathogenic factors of bacteria.
- Study of the bacterial sensitivity to antibiotics.

CULTIVATION OF ANAEROBES

There are several techniques for obtaining anaerobic conditions:

- GasPak anaerobic jar (**anaerostat**) and "**Genbags**" (packets).
- Gas generating boxes ("**Genbox anaer**").
- Reducing agents in culture media.
- Boiling of nutrient broth and layer over it by sterile Vaseline.
- Combined cultivation of aerobes with anaerobes on the same Petri dish.

Media for anaerobes:

- Kitt-Tarozzi medium (cooked meat glucose medium).
- Blood-sugar agar (Zeissler agar).
- Bismuth-sulphite agar (Wilson-Bler medium).
- Thioglycolic medium (medium for control of sterility), etc.

Lecture 2. BACTERIOPHAGES. GENETICS AND ECOLOGY OF MICROORGANISMS

BACTERIOHGES

Bacteriophages (phages) are bacterial viruses. Nomenclature of phages is based on the name of the host which is sensitive to definite phage. Structure of phages — **nucleic acid (DNA or RNA)** and **proteins,** type of symmetry of the phages is **complex** (head has cubical, tail has helical symmetry)**,** but they are **simple viruses** (have no lipid envelope).

The main properties of bacteriophages:

1. Obligate specificity.
2. They more tolerant to the high temperatures than viruses.
3. Stability to the antibiotics, disinfectants and antiseptics.
4. Stability to the high pressure.
5. The proteins of the phages cause the production of antibodies.

Structural components of bacteriophages:

- Head (with NA).
- Collar.
- Tail and tail fibers.
- End plate (base plate).

Morphological types of bacteriophages:

1. Only tail.
2. Only head.
3. With short tail and head.
4. With long unretractile tail and head.
5. With long retractile tail and head.

Types of life cycle of bacteriophages:

- **Virulent (lytic) cycle** → intracellular multiplication of the phage is finished by the lysis of the host bacterium and the release of progeny virions (name of such phages is *virulent phages*).
- **Temperate (lysogenic) cycle** → phage DNA becomes integrated with the bacterial genome and integrated phage genome is called **prophage** (name of such phages is *temperate phages*).

Lytic cycle:

(1) The first step in the replication of the phage is called adsorption. The phage adheres to bacteria by means of its tail fibers.

(2) Phage "injects" its DNA into the bacterial cell ("penetration").

(3) Synthesis of phage proteins and genomes.

(4) Assembly of the phage progeny.

(5) Lysis of the host cell and the release of the progeny.

Lysogenic cycle:

After penetration, the phage DNA integrates into the bacterial chromosome. Temperate viruses usually do not kill the host bacterial cells they infect. These bacteria are called **lysogenic**. The virus in this state is called **prophage**.

Usually it is difficult to recognize lysogenic bacteria because lysogenic and nonlysogenic cells appear identical. But in a few situations the prophage supplies genetic information such that the lysogenic bacteria exhibit a new characteristic (new phenotype), not displayed by the nonlysogenic cell, a phenomenon is called **lysogenic conversion**. Hence, *Corynebacterium diphtheriae* can only produce the exotoxin responsible for the disease (diphtheria) if it carries a temperate virus called phage beta ("toxigenic strains" are *lysogenic*).

Classification of bacteriophages according to the spectrum of their action:

- Polyphages — infect several species of bacteria;
- Monophages — infect one species of bacteria;
- Type phages — infect only a certain phagotype of bacteria.

Practical application of phages in medicine:

1. **Phage typing** (phagotyping) is detection of bacterial phagotype of the pure culture with help of the typical bacteriophages.

2. **Phage therapy** and **phage prophylaxis**.

Phage therapy is the therapeutic use of bacteriophages to treat bacterial infections (salmonellosis, staphylococcal infections, etc.). Bacteriophages are much more specific than antibiotics, so they can hypothetically be chosen to be indirectly harmless not only to the host organism, but also to other beneficial bacteria, such as gut flora, reducing the chances of opportunistic infections. Because phages replicate in vivo, a smaller effective dose can be used. On the other hand, this specificity is also a disadvantage: a phage will only kill a bacterium if it is a match to the specific strain. Consequently phage mixtures ("phage cocktail") are often applied to improve the chances of success, or samples can be taken and an appropriate phage identified and grown. They tend to be more successful than antibiotics where there is a biofilm covered by a polysaccharide layer, which antibiotics typically cannot penetrate.

ORGANIZATION OF BACTERIAL GENETIC MATERIAL

- **Nucleoid** (bacterial chromosome) is a molecule of DNA in cytoplasm which codes vital information for bacteria.
- **Extra-chromosomal genetic factors** (→ additional properties).

Extra-chromosomal factors include:

1. Autonomous factors (**plasmids**) ► its replication is independent.
2. Non-autonomous factors (*integrated* into bacterial nucleoid or plasmids):

✓ **Transposones.**

✓ **IS-elements** ("insertion sequences").

✓ **Temperate bacteriophages** (prophages).

Transposones and IS-elements are nucleotides which can change their localization in the bacterial genetic material — nucleoid or plasmids **(**"jumping genes" of genome), function — change the bacterial genome resulting in the induction of the mutations.

PLASMIDS are small molecules of DNA in a bacterial cytoplasm.

Functions of the plasmids:

1. **Regulatory** (help for nucleoid);

2. **Coding** (introduce new information into bacteria).

Location of plasmids: autonomous and integrated into the nucleoid.

Classification of plasmids:

- **F-plasmid** (→ conjugation in bacteria).
- **R-plasmid** (→ multiple resistance to the antibiotics).
- **Hly-plasmid** (→ synthesis of hemolysins).
- **Ent-plasmid** (→ synthesis of enterotoxins).
- **Plasmids of bacteriocinogenicity** (→ synthesis of the bacteriocins).
- **Biodegradative plasmids.**

F-PLASMID (F-factor)

Sex (**f**ertility) factor provides conjugation in bacteria by forming of *conjugative pili* between the mating bacteria.

F-plasmid contains only **tra-operon** which is need for plasmid transmission (other genes are not presented generally).

Location F-plasmid in bacterial cell:

1. Integrated into bacterial genome (**Hfr-plasmid** — provide **h**igh **f**requency of **r**ecombination between mating bacteria during conjugation).

2. Autonomous F-plasmid.

Cell which have F-plasmid are called male cells (F^+-cells or Hfr-cells). These cells have conjugative pili on their surface. Male cells are the donor cells. Cells without F-plasmid are the female cells (F^--cells), have no conjugative pili. Female cells are recipient cells in conjugation.

R-PLASMIDS

■ Plasmids coding *multiple resistance to antibiotics*.

■ Ways of transmission: by transduction (in Gram-positive bacteria) or by conjugation (in Gram-negative bacteria).

■ Composition:

✓ r-operon + tra-operon (conjugative plasmids).

✓ r-operon (nonconjugative plasmids).

PLASMIDS OF BACTERIOCINOGENECITY (COL-FACTOR)

Bacteriocins are antibiotics' like substances which one bacterial species can produce against closely related species. Ex., *E.coli* can produce colicins against the enteric pathogen.

Properties of bacteriocins:

1. Bacteriocins resemble the antibiotics with narrow spectrum of action.
2. Bacteriocins are causing the destruction of the target cells.
3. After the releasing of bacteriocins the cell will die.

Properties of col-factor:

✓ Rarely integrate into nucleoid;

✓ Usually exist in the repressed state;

✓ Potentially lethal plasmid.

Composition of col-factor:

- tra-operon;

• genes coding synthesis of colicins.

Importance of colicins for medicine: normalization of normal flora of an intestine (preparation is called **colibacterin**).

TYPES OF VARIABILITY OF BACTERIA

1. **Phenotypic** (nonheritable, modification) variability.
2. **Genotypic** (heritable, genetic) variability:

✓ Mutation variability.
✓ Recombinant variability.

3. **SR-dissociation.**

Phenotypic variability is changes affecting only the **phenotype** of bacteria. Modifications of bacteria have certain properties:

► not stable and usually could be lost very quickly;
► may be morphological, biochemical, antigenic, etc.

Mutation variability is changes which occur in the primary structure of DNA molecule. Mutagens are chemical substances or physical factors which cause mutations in DNA structure of bacteria.

Classification of mutations:

✓ Insertions
✓ Deletions
✓ Substitutions
✓ Direct
✓ Reverse
✓ Lethal
✓ Conditionally lethal
✓ Benefit (rare)

✓Spontaneous
✓Inductive
✓Morphological
✓Biochemical
✓Physiological
✓Missense
✓Nonsense

Recombinant variability is changes in DNA structure occurring as a result of integration of the part of DNA of donor cell into DNA of recipient cell. Result of the recombination is a formation of the *recombinant cells* which are cells with new genes and new properties. The genetic information transferred is often beneficial to the recipient. Benefits may include antibiotic resistance, synthesis of virulence factors, xenobiotic tolerance or the ability to use new metabolites.

Forms of the recombinant variability (recombination):

1.Transformation.
2.Transduction.
3.Conjugation.

Transformation is a direct transfer of genetic material from the donor cell to the recipient cell (or uptake of the naked donor DNA fragments by the recipient competent cell).

Factors affecting transformation:

1. DNA size state: Double stranded DNA of works best.
2. Competence of the recipient: the bacteria are said to be *competent* when they are able to take up DNA from outside.

Steps in transformation:

1. Uptake of DNA.

2. *Homologous recombination* between recipient genes and donor genes.

Transduction is a transfer of genetic material from donor cell to recipient cell with help of the *temperate defective bacteriophages*. Often result of transduction is a lysogenic conversion.

Types of transduction:

1. *Generalized transduction* is a transduction in which potentially *any* bacterial gene from the donor can be transferred to the recipient.

2. *Specialized transduction* is a transduction in which only *certain* donor genes can be transferred to the recipient.

3. *Abortive transduction* occurs when the new DNA does not integrate into the chromosome and is eventually lost.

Conjugation is a transfer of genetic material from donor cell to the recipient cell through conjugative pili. During conjugation the donor cell provides a conjugative or mobilizable genetic element that is most often a plasmid or transposon. The transferred DNA can then be integrated into the recipient genome via *homologous recombination*. Conjugation has advantages over other forms of genetic transfer including minimal disruption of the target's cellular envelope and the ability to transfer relatively large amounts of genetic material.

Mating types in bacteria:

✓ F^+-cells and F^- (transfer of single strand of donor F-plasmid)

✓ Hfr-cells and F^- (primarily transfer of donor chromosomal genes)

SR-dissociation is an appearance of R-form and S-form of bacteria. Mechanism is mutation in gene controlling the synthesis of LPS.

Table 2 — Comparison of R-form and S-form of bacteria

S-form	R-form
Smooth colony	**R**ough colony
Usual virulent	Usual nonvirulent (exception *B. anthracis, Y.pestis, M.tuberculosis*)
Capsule and flagella are present	Capsule and flagella are not present
Sensitive to bacteriophages	Less sensitive to bacteriophages
Biochemically active	Biochemically less active
Complete set of antigens	Incomplete set of antigens
More resistant inside of the host to phagocytosis and antibodies	More resistant to unfavorable conditions of environment

R-forms of bacteria are usually more resistant to the unfavorable factors of environment, while S-forms of bacteria are more resistant to immune factors if a host (antibody, complement, phagocytes, etc.).

Molecular-genetic methods applied in microbiological diagnosis:

- Genetic engineering.
- DNA-probe.
- Molecular hybridization.

- Blotting techniques.
- Polymerase chain reaction (PCR).

Polymerase chain reaction (PCR)

PCR is used to amplify a specific region of a DNA strand (the DNA target). **Amplification** is multiple coping of DNA in vitro. Basic PCR set up requires several components and reagents. These components include:

- **DNA template** that contains DNA region (target) to be amplified.
- **Two primers** that are complementary to the ends of DNA target.
- **Taq polymerase** (enzyme for replication).
- **Nucleotides** (dNTPs).

PCR is commonly carried out in small reaction tubes in a thermal cycler. The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction (see below).

Typically, PCR consists of a series of 20–40 cycles. Each cycle consists of several steps:

(1) *Denaturation step*: this step consists of heating the reaction to 94–98 °C for 20–30 seconds. It causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

(2) *Annealing step*: the reaction temperature is lowered to 50–65 °C for 20–40 seconds allowing annealing of the primers to single-stranded DNA template. Polymerase binds to the primer-template hybrid and begins DNA replication.

(3) *Extension/elongation step*: optimum activity temperature is 72 °C used for Taq polymerase. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template. The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified.

PCR in diagnosis of diseases

PCR permits early diagnosis of malignant diseases PCR also permits identification of non-cultivatable or slow-growing microorganisms such as mycobacteria, anaerobic bacteria, or viruses. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes. The high sensitivity of PCR permits virus detection soon after infection and even before the onset of disease. Such early detection may give physicians a significant lead in treatment. The amount of virus ("viral load") in a patient can also be quantified by PCR-based DNA quantitation techniques.

ECOLOGY OF BACTERIA. HUMAN NORMAL FLORA

Ecology is the study of the relationship of organisms to each other and to their environment. Different bacteria are capable of growing in a wide variety of environments but each will grow best in those environments for which it is well adapted.

Ecosystem is biological community including humans and microorganisms.

Population is totality of same species microbes living on the certain territory (biotope).

Biotope is area of the biosphere with same conditions for life.

Biosphere is region of earth inhabited by living organisms.

Bacteria can have a full range of **symbiotic interactions** with their animal hosts.

Symbiosis is living together of two dissimilar organisms (symbionts).

Ecological connections between symbionts**:**

1. **Mutualism** is an association in which both partners benefit.

2. **Parasitism** is a relationship between two organisms in which one organism (parasite) uses other organism as a host for nutrition and replication. **Parasites** are microbes that can multiply inside the host.

Commensalism is a relationship between parasite and host in which they live in a complete harmony without causing any damage for host (ex.: normal flora). Many commensals behave as *facultative pathogens* (or *opportunistic pathogens*) in that they can produce disease when the host resistance is lowered.

Obligate parasitism is a relationship in which parasite cause damage to the host. Obligate parasites are mainly *obligate pathogens.*

Steps of the development of parasitism:

1. **Saprophytes:**

They are free-living microbes that subsist on dead or decaying organic matter in nature (soil, water). They are generally incapable to multiply in the living tissues. Exceptionally, some saprophytes (like *Bacillus subtilis*) may infect only the immunocompromised persons.

2. **Facultative parasites** (can live a long time as saprophytes);

3. **Obligate parasites** (live mainly in a host):

- Facultative intracellular **parasites**.
- Obligate intracellular **parasites.**

Sanitary microbiology

Soil contains a large variety of microorganisms that are able to degrade or chemically modify organic and inorganic molecules. Soil environment includes different forms of life like bacteria (most important — actinomycetes), algae, fungi (Candida, Aspergillus, Penicillium, Rhizopus, and Mucor are most common), protozoa, nematodes, worms and other eukaryotic organisms.

Air contains Sarcina, Bacilli, actinomycetes, fungi.

Aquatic environment contains aerobic cocci, Leptospira, Pseudomonades, Proteus, anaerobic bacteria.

NORMAL FLORA

Normal flora (normal microbial flora, microflora, microbiota) is a group of the microorganisms that colonize the body surfaces but does not usually cause a disease.

- May be residents (constant) and transients (occasional).
- Constant for each species.
- 400 species of normal flora have been recognized.

Ecological position of normal flora: commensals and parasites.

Localization of a normal flora – skin, mucous, conjunctiva, upper respiratory tract, lower urogenital tract, almost all gastrointestinal tract.

Composition of normal flora:

Skin → Staphylococci, Diphtheroids, Candida, Propionibacteria.

Upper respiratory tract → Streptococci, Staphylococci, Diphtheroids (larynx, trachea, bronchi, lung do not contain normal flora).

Oral cavity normal flora → Staphylococci, Streptococci, Candida, Lactobacteria, Spirochetes, Neisseria, Peptostreptococci, Bacteroides.

Colon normal flora → Bacteroides and Bifidobacteria (96–99 %), *E.coli*, Enterococci, Clostridia, Candida, Proteus and also Enterobacter, Citrobacter, Klebsiella.

Female genital tract → Lactobacilli, some Streptococci, Candida.

Male urethra is relatively sterile.

Blood, CSF, synovial liquid are sterile.

Role of normal flora:

- Prevent colonization of body by other pathogens.
- Stimulation of immune system (normal flora have related antigens with pathogens).
- Intestinal flora produce vitamins (B, K).
- Some species produce antibiotic substances (colicins).
- Digestion of food in the colon.
- Neutralization of endogenous or exogenous toxic substances.

Normal flora as pathogens

Most species of normal flora are the facultative pathogens, some species are nonpathogenic, and some ones are the obligate pathogens.

Normal flora can produce infections (can be named so: opportunistic infections, endogenous infections, autoinfections, secondary infections, etc.) at certain conditions: immunocompromised status, antibacterial therapy, penetrating trauma, etc.

Disbacteriosis (disbiosis) is a qualitative and quantitative misbalance of the normal flora. This can lead to an overgrowth of one or more of the pathogenic microorganisms (bacteria or fungi) which then may damage some of the other smaller beneficial ones.

Disbiosis is the most prominent in the digestive tract or on the skin, but can also occur on any exposed surface or mucous membrane such as the vagina, lungs, mouth, nose, sinuses, ears, nails, or eyes.

It has been associated with the different illnesses, like inflammatory bowel disease and chronic fatigue syndrome.

Balance of normal flora is disturbed by such diverse things as repeated and inappropriate antibiotic exposure or alcohol misuse.

Treatment of disbiosis:

✓ **Probiotics** (ex.: lactulosa).

✓ **Eubiotics** (ex.: colibacterin, lactobacterin).

Lecture 3. ANTIMICROBIAL ACTIONS. CHEMOTHERAPY OF THE BACTERIAL INFECTIONS. THE FOUNDATIONS OF THE THEORY ABOUT INFECTION

MICROBIAL DECONTAMINATION is complete or partial removal of microorganisms from the objects of surrounding environment or from the human organism with use of the factors causing direct damage of microorganisms.

Types of decontamination:

- Decontamination of the objects (sterilization and disinfection).
- Decontamination of live organisms (antiseptics and chemotherapy).

Antimicrobial agents:

Bactericides ► killing of microorganisms

Bacteriostatics ► inhibition of bacterial multiplication

STERILIZATION

Sterilization is a complete removal or killing of all microorganisms (vegetative cells and spores) on the objects of environment. Objects after sterilization are called **sterile**.

Types of sterilization:

1. Sunlight (spontaneous).
2. Drying.
3. Dry heat sterilization (flaming, incineration, hot air).
4. Moist heat sterilization (pasteurization, boiling, autoclaving).

Autoclaving is a sterilization by hot water steam with use of high pressure; T of steam = 110–140 °C, in autoclave).

Fractional sterilization (tyndallisation) is sterilization by the flowing steam (30 minutes under the temperature of 100 °C, with several intervals for one day to cool the material and to enable the spores to germinate).

5. Filtration (candles, asbestos pads, membranes);
6. Radiation (gamma rays or UV rays);
7. Ultrasonic and sonic vibrations;
8. Chemical sterilization: use formaldehyde, ethylene oxide.

DISINFECTION AND ANTISEPTIC

Disinfection is elimination or reduction of the definite group of the pathogenic microorganisms usually with use of chemical agents (substances) which called ***disinfectants.*** Other type of disinfection is physical (mechanical, thermal factors, radiation/ UVR, etc.).

Disinfectants are bactericidal products used to kill the microbes on the inanimate objects or surfaces (may be sporostatic, but not sporocidal).

An **antiseptic** is inhibition of the growth (rare killing) of the disease-causing microbes on the intact and injured skin and mucous surfaces usually with use of chemical agents which called ***antiseptics***.

Antiseptics are chemical disinfectants which can be safely applied to skin or mucous membrane and are used for prevention infection by inhibiting the growth of the disease-causing bacteria.

Common disinfectants and antiseptics:

1. Ethanol (70–80%).
2. Formaldehyde, glutaraldehyde.
3. Phenol compounds (carbolic acid).
4. Halogens (chlorine, iodine and their derivatives).
5. Metallic salts (salts of copper, mercury, $AgNO_3$).
6. Oxidants (ozone, H_2O_2, $KMnO_4$).
7. Surfactants (detergents, surface-active agents) included cationic, anionic, nonionic compounds.
8. Quaternary ammonium compounds.
9. Aniline dyes (brilliant green, crystal violet, malachite green).
10. Alkylamines, guanidines, etc.

ASEPSIS

Asepsis is creation of the zone free from any microorganisms in the next places: patient's areas, rooms where are medical manipulations, clinical laboratories (creation of the aseptic conditions).

Aseptic is characterized by the absence of the pathogenic microbes.

Septic is characterized by the presence of pathogenic microbes in the living tissues.

Methods of asepsis are direct (sterilization, disinfection, antiseptics) and indirect (separation, masks, gloves, work in boxes, etc.).

ANTIMICROBIAL AGENTS

1. **Antiseptics**: mercurials, silver nitrate, iodine solution, alcohols.
2. **Disinfectants**: chlorine, chlorine compounds, lye, copper sulfate, quaternary ammonium compounds.
3. **Preservatives**: static agents used to inhibit the growth of microorganisms, most often in foods.
4. **Chemotherapeutic preparations (CTPs)**: antimicrobial agents of synthetic origin useful in the treatment of bacterial, viral diseases and tumors.
5. **Antibiotics**.

Chemotherapy is treatment of infection and tumors with help of CTPs which are selectively kill or inhibit infectious agents or tumor cells inside of a host body. CTPs are medical preparations which are used for chemotherapy. They are classified into antibiotics (of natural, synthetic, semisynthetic origin) and synthetic CTPs. Synthetic CTPs are absent in nature and have no natural analogs.

The main groups of CTPs are sulphonilamides, nitrophurans, nitroimidasoles, quinolones, organic and inorganic compounds of S, As, Cu, Hg, etc.), antituberculous preparations (isoniazid), antiviral preparations (acyclovir), antitumoral preparations, etc.

ANTIBIOTICS

Antibiotics are chemotherapeutic agents of natural, semisynthetic or synthetic origin that in low concentrations are able to cause inhibition or killing sensitive to them microorganisms or tumor cells inside of macroorganism.

Classification of antibiotics according to groups of sensitive microorganisms:

1. Antibacterial.
2. Antiviral.
3. Antifungal.
4. Antitumoral.
5. Antiprotozoal.

Classification of antibiotics according to source of receiving:

- Fungi (Penicillium).
- Actinomycetes (80 % of antibiotics are produced by Streptomyces).
- Bacteria (Bacillus and Pseudomonas).
- Synthetic antibiotics.

Classification of antibiotics according to their effect:

- Bacteriostatic (macrolides, tetracyclines, etc.).
- Bacteriocidal (beta-lactams, aminoglycosides, etc.).

Classification of antibiotics according to spectrum of action:

1. Narrow spectrum.
2. Moderate spectrum.
3. Broad spectrum.

Classification of antibiotics according to mechanism of action on bacteria:

a) ***Cell wall synthesis inhibitors****:*
   - ✓ Beta-lactams (ex.: penicillins and cephalosporins).
   - ✓ Carbapenems.
   - ✓ Monobactams.
   - ✓ Glycopeptides.

b) ***Cell membrane inhibitors:***
   - ✓ Polymyxin.
   - ✓ Nystatin.
   - ✓ Imidazole.

c) ***Protein synthesis inhibitors:***
   - ✓ Tetracyclines (ex.: tetracycline, doxycycline).
   - ✓ Aminoglycosides (ex.: streptomycin, gentamycin).
   - ✓ Chloramphenicol (laevomycetinum).
   - ✓ Macrolides (ex.: erythromycin).

d) ***Nucleic acid synthesis inhibitors:***
   - ✓ Quinolones and fluorquinolones (ex.: nalidixic acid).
   - ✓ Rifamycines (ex.: rifampicin).

e) ***Competitive inhibitors*** (or anti-metabolites, growth factors analogs → they are specifically inhibiting essential metabolic pathways in the bacterial pathogen).

✓ Ex.: Sulfonilamides (trimethoprim or biseptol).

Characteristics of clinically-useful antibiotics:

1. It should have a wide spectrum of activity with the ability to destroy or inhibit many different species of pathogenic organisms.
2. It should be nontoxic to the host and without undesirable side effects.
3. It should be nonallergenic to the host.
4. It should not eliminate the normal flora of the host.
5. It should be able to reach the part of the human body where the infection is occurring.
6. It should be inexpensive and easy to produce.
7. It should be chemically-stable (have a long shelf-life).
8. Microbial resistance is uncommon and unlikely to develop.

Principles of rational antibiotic therapy:

1. *Microbiological principle* (to take into account etiology of infection).
2. *Pharmacological principle* (→ pharmacodynamics and pharmacokinetics of the antibiotic, its possible interaction with other drugs).
3. *Clinical principle* (→ state of patient).
4. *Epidemiological principle* (→ the microbial resistance to a given antibiotic in this country, region, hospital, etc.).

Side effects of antibiotic therapy:

- Toxicity (hepatotoxicity, endotoxic shock, etc.).
- Disbacteriosis (indiscriminate use of broad-spectrum antibiotics can lead to superinfection by opportunistic microorganisms, such as *Candida* and *Clostridium difficile*, when the body's normal flora is destroyed).
- Depression of immunity.
- Allergy (anaphylactic shock).
- Mutagenic reactions on fetus (terratogenic effect).
- L-transformation.
- Development of antibiotic resistance.

Problem of drug resistance

Bacterial pathogen is able to develop or acquire resistance to antibiotic, then that substance becomes useless in the treatment of infectious disease caused by that pathogen. So as pathogens develop resistance, we must find new (different) antibiotics to fill the place of the old ones in treatment regimes.

Types of resistance:

- *Natural resistance* (characteristic for all isolates of species, genus, family, certain groups of bacteria, etc.; for example, anaerobes are naturally resistant to aminoglycosides; intracellular parasites are resistant to beta-lactams because these antibiotics do not enter cells; mycoplasma is also resistant to beta-lactams because its lacks cell wall).
- *Acquired resistance* (characteristic for certain strain/strains, for example MRSA — **M**ethicillin **R**esistant ***S****taphylococcus* ***a****ureus*).

Problematic bacteria are bacteria which resistant to the antibiotics (→ "problematic patients"), have often multiple resistance; produce the nosocomial (intrahospital) infections.

Examples of the most distributive **nosocomial agents:**

✓ Enterobacteria (Salmonella).

✓ *Pseudomonas aeruginosa*.

✓ Staphylococci (MRSA).

✓ Enterococci (VRE – **V**ancomycin **R**esistant **E**nterococci).

Genetic mechanisms of drug resistance:

• *Chromosomal resistance* develops as a result of spontaneous ***mutation*** in a locus that controls the sensitivity to a given drug. \

• *Extrachromosomal resistance.* Bacteria are able to exchange genes in nature by three processes: conjugation, transduction and transformation. Genetic recombination can follow the transfer of DNA from one cell to another leading to the emergence of a new genotype (recombinant). It is common for DNA to be transferred as *plasmids* between mating bacteria. Since bacteria usually develop their genes for *multiple drug resistance* (for several antibiotics) on ***R-plasmids*** (or resistance transfer factors/RTFs), they are able to spread drug resistance to other strains and species during genetic exchange. Thus, plasmids determine resistance to penicillin by carrying genes or the formation of β-lactamases. ***Transposons*** provide the resistance to a one antibiotic.

Nongenetic mechanisms of drug resistance*:*

• Microorganisms produce ***enzymes*** that destroy the active drug. E.g. Staphylococci resistant to natural penicillins due to their production of *beta-lactamases*.

• Microorganisms change their ***permeability*** to the drug (reduced *influx*, increased *efflux* of antibiotics).

• Microorganisms develop an altered (changeable) structural target for the drug. E.g. resistance to penicillin may be function of the loss of penicillin-binding proteins (PBPs) in the bacterial cell wall.

• Microorganisms develop altered metabolic pathways that bypass the reaction inhibited by the drug. Some sulfonamide-resistant bacteria do not require paraaminobenzoic acid (PABA) but, like mammalian cells, can use folic acid.

Antibiotics sensitivity (susceptibility) tests:

1. *Minimum inhibitory concentration tests*:

► Broth dilution test.

► Agar dilution test.

► Method of E-tests.

2. *Disk diffusion test* (or method of the standard disks).

Mechanisms to reduce antibiotic resistance:

1. Control, reduce or cycle antibiotic usage.

2. Improve hygiene in hospitals and among hospital personnel and reduce movement of patients to eliminate the dissemination of resistant microbes within hospitals.

3. Discover or develop new antibiotics.

4. Modify existing antibiotics chemically and study mechanisms of resistance.

5. Develop inhibitors of antibiotic-destroying enzymes (beta-lactamase inhibitors such as clavulanic acid, sulbactam).

THE FOUNDATIONS OF THE THEORY ABOUT INFECTION

Infection is multiplication of infectious agent within the body.

Multiplication of a normal flora is generally not considered as infection (but at certain conditions normal flora produce opportunistic infections); on other hand, multiplication of the pathogenic bacteria – even is person is asymptomatic — is deemed an infection.

Carrier is a person with the asymptomatic infection that can be transmitted to another person.

Parts of infection:

1. Microorganism.
2. Macroorganism.
3. Factors of environment.

INFECTIOUS DISEASE is rare, terminal consequence of the infection.

Infection may imply colonization, multiplication, invasion of a pathogen, but infectious disease is used to describe an infection that causes significant overt damage to the host.

Table 3 — Periods of infection disease

Periods	Pathogen	Symptoms	Host is contagious?	Immune response
Incubatory	Adhesion	Without	No	No
Predromal	Colonization	Nonspecific	Probably	No
Clinical manifistations	Multiplication	Specific symptoms	Yes	Ig M then Ig G
Recovery Complete Incomplete (carrier state)	Death or slow multiplication	Normalization	Release during carrier state only	Ig G

PATHOGENICITY AND VIRULENCE

Pathogenicity is an ability of the infectious agent to cause a disease (it is genetic feature of the microbial species).

Classification of bacteria according to pathogenicity:

1. Nonpathogen is a microorganism that does not cause the disease (they are usually components of indigenous microflora or saprophytes).

2. Opportunistic pathogen (facultative pathogen) is an agent capable of causing the disease only when host's resistance is impaired (patient is "immunocompromised").

3. Pathogen (obligate, strict pathogen) is a microorganism capable to cause of the disease even in "healthy" individuals.

Opportunistic pathogens can cause the opportunistic infections or diseases only in certain conditions like:

✓ Decrease of immunity.

✓ High infectious dose.

✓ Change the site of localization due to penetrating trauma or medical manipulations (e.g., fecal flora enters urinary tract; skin flora binds with catheters and so on).

Virulence (degree of pathogenicity) is quantitative ability of an agent to cause disease (it is individual feature of the strain).

Virulence can be increased or decreased in vivo and in vitro (ex.: avirulent strain is strain which lack virulence).

Pathogenic factors (or virulence factors) are factors that are produced by a microorganism and evoke disease, include:

1. Factors of adhesion and colonization.
2. Factors of invasion.
3. Toxins.

Adhesion (adherence, attachment) is process by which bacteria stick to the surface of host cells. Once bacteria have entered the body, *adhesion is initial step in the infectious process.*

Colonization is multiplication of bacteria on the body surfaces.

Factors of adhesion and colonization:

- Capsules.
- Adhesins (pili/fimbria) binding with receptors of host.
- LPS and proteins of outer membrane (in Gram-negative bacteria).
- Teichoic acids (in Gram-positive bacteria).

Biofilm formation

A *biofilm* is any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of *extracellular polymeric substance* (EPS). Biofilm EPS which is also referred to as slime, is a polymeric conglomeration generally composed of extracellular DNA, proteins, and polysaccharides. Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium.

Biofilms have been found to be involved in a wide variety of microbial infections (urinary tract infections, catheter infections, middle-ear infections, formation of dental plaque, coating contact lenses, endocarditis, and infections of permanent indwelling devices such as joint prostheses and heart valves). More recently it has been noted that bacterial biofilms may impair cutaneous wound healing and reduce topical antibacterial efficiency in treating infected skin wounds. Biofilms can also be formed on the inert surfaces of implanted devices such as catheters, prosthetic cardiac valves and intrauterine devices. Biofilms are less susceptible to antibiotics; provide treatment failure, survival of bacteria and predispose to recurrent bacterial infections.

Invasion is ability of pathogen to spread in the host tissues after establishing of infection.

Factors of invasion (invasions):

- **Special proteins** (internalin, outer membrane proteins — OMPs);
- **Different enzymes** (hayluronidase, neuraminidase, fibrinolysin, coagulase, protease, nuclease, lipase, DNA-ase, RN-ase).

Toxigenicity is ability of pathogen to produce toxins. Bacteria can have two types of toxins — **exotoxins** and **endotoxins**.

Classification of exotoxins due to affected cells:

- Neurotoxins (affect neurons).
- Cytotoxins (block of the protein synthesis in many different cells).
- Enterotoxins (affect enterocytes).
- Hemolysins (increase of membrane permeability and damage of RBCs).

Classification of exotoxins due to mechanism of action:

1. *AB-toxins* (binding subunit B and catalytic active subunit A).
2. *Membrane toxins* (formation of the pores in the different cells and cause their lysis, like hemolysins and leucocidins).
3. *Superantigenes* (stimulate macrophages and T-lymphocytes to produce excessive amounts of harmful cytokines).

Effects of endotoxin:

1. Pyrogenicity (fever) or hypothermia;
2. Activation of host defense (complement activation, activation of macrophages and interferon synthesis).
3. Depression in blood pressure.
4. Endotoxic shock.

Table 4 — Characteristics of endotoxin and exotoxin

Property	Exotoxin	Endotoxin
1. Bacterial source	Gram-negative and Gram-positive bacteria	Only Gram-negative bacteria
2. Location	Actively secreted by bacterial cells (extracellular)	Form part of the cell wall (outer membrane)
3. Composition	Protein	LPS
4. Stability	Heat labile	Heat stable
5. Antigenicity	Highly antigenic; stimulate formation the Ab-antitoxins	Weakly antigenic
6. Specificity	Specific effect for each exotoxin	Action common to all endotoxins
7. Enzymatic activity	Usually	No
8. Pyrogenicity	Occasionally	Yes
9. Interaction with specific antibody	Can be neutralized by antibodies	Neutralization by Ab is ineffective
10. Obtaining	Separation from bacterial culture by physical means as filtration	Obtained only by bacterial cell lysis
11. Toxoid (anatoxin)	Yes	No
12. Activity	Active in very minute doses	Active only in very large doses

Secretion systems

Secretion in bacterial species means the transport of effector molecules (proteins, enzymes or toxins) across the cell wall. Secretion is a very important mechanism in bacterial functioning and operation in their natural surrounding environment for adaptation and survival.

Gram-negative bacteria have two membranes, thus making secretion topologically more complex. There are at least six specialized secretion systems in Gram-negative bacteria. The most interesting is **type III secretion system** (T3SS). It is homologous to bacterial flagellar basal body. It is like a *molecular syringe* through which a bacterium (e.g. Salmonella, Shigella, Yersinia and Vibrio) can inject proteins into eukaryotic cells.

Detection of virulence:

✓ *Direct revealing*: biological probe on animals;
✓ *Indirect revealing*: detection of enzymes of virulence in vitro.

Measurement of virulence:

- Dlm (*Dosis letalis minima*).
- LD 50.
- Dcl (*Dosis certa letalis*).
- ID (infectious dose).

CLASSIFICATION OF INFECTIONS:

On the base of number of pathogens:
✓ Mono-infection.
✓ Mixed infection (co-infection).

On localization of pathogen:
✓ Localized.
✓ Generalized (bacteremia, sepsis, virusemia, endotoxemia, toxenemia, toxic shock).

On source of infection:
✓ Anthronosis.
✓ Zoonosis.
✓ Sapronosis .

On place of beginning:
✓ Intrahospital (nosocomial).
✓ Out the hospital (natural).

On duration:
✓ Acute.
✓ Subacute.
✓ Chronic.
✓ Fulminant.
✓ Slow.

On origin:
✓ Exogenic.
✓ Endogenic (autoinfection).

Repeated infections:
✓ Re-infection.
✓ Super-infection.
✓ Secondary infection (opportunistic).
✓ Relapse.

On spreading:
✓ Sporadic case.
✓ Endemy.
✓ Epidemy.
✓ Pandemy.

On clinical manifestations:
✓ Manifest.
✓ Inapparent (subclinical).
✓ Atypical/Typical.
✓ Severe/Mild.
✓ Microbocarriage.
✓ Persistent (chronic or latent).

MECHANISMS OF TRANSMISSION OF INFECTIOUS DISEASES

- **Fecalo-oral mechanism of transmission:**

Modes of transmission:

✓ Food-borne (alimentary).

✓ Water-borne.

✓Contact (hand-borne).

✓ Portal of entry — GIT.

- **Aerogenic mechanism (inhalation, air-borne):**

Modes of transmission:

✓ By droplets (human-to-human, during sneezing, cough, speech).

✓ By dust particles (soils, dried animals excrements) with adsorbed pathogens or their spores.

✓ Portal of entry — URT.

- **Blood-borne mechanism:**

Modes of transmission:

✓ Transmissive (bites of vectors-insects).

✓ Parenteral (by inoculation, medical manipulations, blood- transfusion).

✓ Sexual.

✓ Portal of entry — blood.

- **Contact mechanism:**

Modes of transmission:

✓ Contact through animal bites.

✓ Direct contact (human-to-human, animal-to-human).

✓ Indirect contact (with help of inanimate objects).

✓ Sexual contact (contact of mucous).

✓ Portal of entry — skin surfaces and mucous membranes (mainly injured), conjunctiva.

- **Congenital (vertical) mechanism:**

Modes of transmission:

✓ Transplacental (prenatal).

✓ Portal of entry — tissues of fetus.

✓ Also during birth delivery and breast feeding.

Lecture 4. IMMUNOLOGY. IMMUNITY. NATURAL OR NONSPECIFIC RESISTANCE

Branches of immunology:

- General immunology.
- Immunoprophylaxis (vaccinology).
- Immunotherapy.
- Immunodiagnostics.
- Allergology.
- Immunology of transplantation and malignancy.
- Immunohematology.
- Immunopharmacology.

IMMUNITY is a reaction of the body against any foreign material (ability to recognize and eliminate of genetically foreign antigen).

Classification of the types of immunity:

- On the base of mechanism:
  - ✓ Humoral (AMI or **a**ntibody-**m**ediated **i**mmunity).
  - ✓ Cellular (CMI or **c**ell-**m**ediated **i**mmunity).
- On direction:
  - ✓ Antibacterial (antimicrobial).
  - ✓ Antitoxic.
  - ✓ Antiviral.
  - ✓ Antitumoral.
  - ✓ Autoimmunity.
- On base of origin:
  - ✓ Innate (inborn, natural, nonspecific).
  - ✓Adaptive (acquired, specific).
- On participation of own immune system:
  - ✓ Active.
  - ✓ Passive.

Innate immunity (nonspecific) is the natural resistances with which a person is born. It provides resistances through several physical, chemical and cellular approaches. Microbes first encounter the epithelial layers, physical barriers that line skin and mucous membranes. Subsequent general defences include secreted chemical signals (cytokines), antimicrobial substances, fever, and phagocytic activity associated with the inflammatory responses. The phagocytes express cell surface receptors that can bind and respond to common molecular patterns expressed on the surface of invading microbes. Through these approaches, innate immunity can prevent the colonization, entry and spread of microbes.

Table 9 — Comparison between innate and adaptive immunity

Innate immunity	Adaptive immunity
Response is nonspecific	Pathogen and antigen specific response
Exposure leads to immediate maximal response	Lag time between exposure and maximal response
Systemic, cell-mediated and humoral components	Cell-mediated and humoral components
No immunological memory	Exposure leads to immunological memory

Adaptive immunity (acquired) is often divided into two major types depending on how the immunity was introduced. "Naturally acquired immunity" occurs through contact with a disease causing agent, when the contact was not deliberate, whereas "artificially acquired immunity" develops only through deliberate actions such as vaccination. Both naturally and artificially acquired immunity can be further subdivided depending on whether immunity is induced in the host or passively transferred from an immune host. "Passive immunity" is acquired through transfer of antibodies or activated T-cells from an immune host whereas "active immunity" is induced in the host itself by antigen. The table below summarizes these divisions of immunity

Table 10 — Comparison between active and passive immunity

Active immunity	**Passive immunity**
Formation of own antibodies	Transferred by ready-made antibodies (or T-cells)
Long-lasting (months and years)	Nonlong-lasting (several weeks)
Time is need for development of effect (several weeks)	Immediate effect (neutralization of microbes and their exotoxins)

Innate (inborn) immunity in general is protection against infections caused by the pathogens ("1-st line of defense").

Levels of an innate immunity:

►SYSTEM LEVEL (nonspecific resistance):

• Anatomical defenses.

• Microbial antagonism (normal flora and bacteriocins).

• Inflammation.

►CELLULAR LEVEL (cellular nonspecific immune factors):

• Phagocytes.

• Null cells (NK-cells, K-cells).

►HUMORAL LEVEL (humoral nonspecific immune factors):

• Tissue bactericidal substances.

Factors of nonspecific resistance:

1. Mechanical factors:

✓ Intact skin and mucous (fatty acids, sweet, normal flora of skin).

✓ Movement the secretions of the organs — urine, saliva, tears, sputum, feces and elimination of microbes with them.

✓ Ciliated epithelium (also eliminates the microbes).

2. Chemical and biochemical factors:

✓ Sweet (low pH and fatty acids).

✓ Lysozyme is present in most biological liquids (tears, serum, saliva, etc.) → break down of cell wall; it is produced by monocytes and tissue macrophages.

✓ Rapid pH change from stomach to upper intestine.

✓ Low pH of vagina.

3. Physiological factors (coughing, sneezing, vomiting, diarrhea, etc.).

Inflammation is pathophysiological reaction of tissue to the pathogen that accumulates all antimicrobial factors in the site of infection.

Signs of inflammation: heat, swelling, pain, redness.

The overall effect of an inflammatory reaction is to recruit various cells and components to the actual site of microbial invasion. Many of these cells and plasma components have a direct role in defense against the intruding microorganism. These include neutrophils (phagocytes which engulf and destroy the microbes); macrophages and lymphocytes which are the cells necessary to initiate immunological responses against the pathogen; pre-existing antibodies which can neutralize microbial pathogens or their toxins; and plasma components such as lysozyme, complement and fibrin, which have a variety of antimicrobial activities.

As a result of infection or tissue damage, complement is activated leading to the release of complement fragments that can activate local mast cells leading to the re-lease of vasoactive chemicals such as leukotrienes, prostaglandins and histamine. They have an effect on the local endothelium increasing the expression of a range of adhesion molecules, facilitating the immobilization of leukocytes. Prostaglandins cause blood vessel dilatation and enhance the effects of histamine and bradykinin on vascular permeability. Leukotrienes stimulate the migration of leukocytes into the tissues.

PHAGOCYTES

Professional phagocytes:

1. **Mononuclear phagocytes** (blood **monocytes** and tissue **macrophages**).

2. **Polymorphonuclear leucocytes (granulocytes)** or **microphages** (especially, neutrophils are actively phagocytic and form the predominant cell type in acute inflammation, they are forming pus).

Neutrophils have short life span. They circulate in the blood for 6–7 hours, and then migrate through the endothelial cell junctions and reside in tissue spaces where they live only for few days and do not multiply. Neutrophils are the most abundant of the leukocytes, normally accounting for 54–75 % of the WBCs. Neutrophils may increase two- to three-fold during active infections. The nucleus of a neutrophil is segmented into 3–5 connected lobes, hence the name polymorphonuclear leukocyte. Because of the granules, they are considered as one of the granulocytes. Specific granules are present in abundance and contain proteolytic enzymes such as lysozyme, collagenase and elastase.

Monocytes have rounded or kidney-shaped nuclei with finely granular cytoplasm, and have half-life of 3 days in circulation. Monocytes normally make up 2–8 % of the WBCs. Once monocytes leave circulation and enter tissue, they are called *macrophages*. There are two types of macrophages, one that wander in the tissue spaces and the other that are fixed to vascular endothelium of liver, spleen, lymph node and other tissue. *Tissue macrophages* survive for months and can multiply. Macrophages present in different organs have been given different names. They are **histiocytes** (in tissue), **Kupffer cells** (in liver), **alveolar macrophages** (in lungs), **peritoneal macrophages** (in peritoneum), **microglial cells** (in brain), **mesangial cells** (in kidneys) and **osteoclasts** (in bone). Some macrophages develop abundant cytoplasm and are called epitheloid cells. Macrophages can fuse to form multi-

nucleated giant cells. Some mononuclear cells differentiate into dendritic cells. Functions of macrophage include killing of microbes, virus infected cells, tumor cells, secretion of immunomodulatory cytokines, antigen processing and presentation to T cells. Macrophages respond to infections as quickly as neutrophils but persist much longer; hence they are dominant effector cells in the later stage of infection.

Macrophages express many surface receptors (receptors for complement, for immunoglobulins, CD14, and MHC class II).

Functions of macrophages:

1. *Phagocytosis* (primary function of macrophages).

2. *Antigen presentation* for T-cells (T-cells can recognize only processed and presented microbial Ag in connection with MHC).

3. *Secretary function* (they secrete a number of biologically active substances, including hydrolytic enzymes, tumor necrosis factor, colony stimulating factor, interleukine-1, etc.).

PHAGOCYTOSIS

Phagocytosis is intracellular killing of microbes by the phagocytic cells (*intracellular nonspecific cytotoxicity*).

Steps of phagocytosis:

(1) **Delivery** of phagocytes to the site of an infection *Diapedisis*: the migration of cells across vascular walls which is initiated by the mediators of inflammation (histamine, prostaglandins, etc.). *Chemotaxis* is movement of the cells in response to a chemical stimulus. A number of chemotactic factors (chemoattractants) have been identified, both for neutrophils and monocytes. These include bacterial products, cell and tissue debris, and components of the inflammatory exudate derived from complement (C3a and C5a).

(2) Phagocytic **adherence** (with help of opsonins like IgG, IgM, C3b and C5b). OPSONIZATION is coating of the particles (microbes) to improve adherence and engulfment of them.

(3) **Engulfment** of microbe and formation of phagocytic vacuole – phagosome. Membranes of the phagosome and lysosome actually fuse resulting in a digestive vacuole called the phagolysosome. It is within the phagolysosome that killing and digestion of the engulfed microbe takes place.

(4) **Metabolic stimulation** and **killing** of the microbes. Dead microbes are rapidly degraded in phagolysosomes to low molecular-weight components. Various hydrolytic enzymes are involved including lysozyme, proteases, lipases, nucleases, and glycosylases. Neutrophils die and lyse after extended phagocytosis, killing, and digestion of bacterial cells. This makes up the characteristic properties of pus. A content of phagolysosome is eliminated by exocytosis.

Mechanisms of intracellular killing:

► Oxigen-indipendent mechanism: fusion phagosome and lysosome and killing of a pathogen inside the lysosome by lysozyme, lactoferrin, cationic proteins, hydrolytic enzymes and other bactericidal substances.

► Oxigen-dipendent mechanism:

• Oxygen use is increased.

• Accumulation of toxic products.

• "Respiratory explosion" of microbes.

Then microbial antigens are inserted into the receptors on the surface of macrophage (MHC class II) for their **presentation** to T-lymphocytes to activate specific immune response.

➢ *Bacteria can avoid the attention of phagocytes in a number of ways*:

1. Pathogens may invade or remain confined in regions inaccessible to phagocytes (e.g. the lumens of glands, the urinary bladder are not patrolled by phagocytes).

2. Some pathogens are able to avoid provoking an overwhelming inflammatory response. Without inflammation the host is unable to focus the phagocytic defenses.

3. Some bacteria or their products inhibit phagocyte chemotaxis.

4. Some pathogens can cover the surface of the bacterial cell with a component which is seen as "self" by the host phagocytes and immune system. Such a strategy hides the antigenic surface of the bacterial cell. For example, *Staphylococcus aureus* produces cell-bound coagulase which clots fibrin on the bacterial surface.

5. Classical examples of antiphagocytic substances on the bacterial surface include: polysaccharide capsules of pneumococci and meningococci, O-polysaccharide associated with LPS of *E.coli*.

6. Some bacteria survive inside of phagocytic cells, in either neutrophils or macrophages. Bacteria that can resist killing and survive or multiply inside of phagocytes are considered *intracellular parasites*. In this case, the environment of the phagocyte may be a protective one, protecting the bacteria during the early stages of infection or until they develop a full complement of virulence factors. The intracellular environment protects the bacteria against the activities of extracellular bactericides, antibodies, drugs, etc. Some bacteria, such as Brucella and Mycobacteria, resist intracellular digestion and may actively multiply inside the phagocytic cells. Phagocytosis in such instances may actually help to disseminate infection to different parts of the body.

NK CELLS (LARGE GRANULAR LYMPHOCYTES):

• Large lymphocytes containing azurophilic granules in the cytoplasm.

• NK cells derive form bone marrow but don't require thymus for development. NK cells are so called because they kill variety of target cells (such as tumor cells, virus-infected cells, and transplanted cells) without the participation of MHC molecules (nonspecific cytotoxicity). They can kill target cell without a need for activation unlike cytotoxic T-lymphocytes. Hence they mediate a form of natural (innate) immunity.

• Distribution: they account for 10–15 % of blood lymphocytes. They are rare in lymph nodes and don't circulate through lymph.

• Surface markers: NK cells lack any surface immunoglobulins, TCR or CD4 makers; instead they have CD16 (Immunoglobulin Fc-receptor) and CD56.

Approximately 50 % of human NK cells express only one form of CD8. Other receptors include IL-2R, CD2, ICAM-1 and LFA

• Functions: NK cells are activated by recognition of antibody-coated cells (name of these NKs are K-cells, they provide ADCC — **a**ntibody-**d**ependent **c**ell-mediated **c**ytotoxicity), virus infected cell, cell infected with intracellular bacteria and cells lacking MHC I proteins. Activation of NK cell results in cytolysis of target and cytokine secretion but no clonal expansion. Interestingly, NK cells are inhibited on contact with MHC I proteins.

NK cells also participate in Graft vs Host reaction in recipient of bone marrow transplants. NK cells can be activated by IL-2 so that their cytotoxic capacity is enhanced. Such cells are called **l**ymphokine **a**ctivated **k**iller cells (LAK) and have been used to treat tumors. LAK cells have enhanced cytolytic activity and are effective against wide range of tumor cells.

TISSUE BACTERIOCIDES:

- Complement system.
- Lysozyme.
- Peroxidase.
- Interferons — IFN (antiviral proteins).
- ß-lysins (serum protein for lysis of CPM).
- Antimicrobial peptides (defensins).
- Interleukins (IL).
- Acute phase protein (C-reactive protein — CRP).
- Lactoferrin (iron-binding protein).

Interferons (IFN) are classes of antiviral proteins produced by certain animal cells after viral stimulation. IFNs belong to the large class of proteins known as cytokines; molecules used for communication between cells to trigger the protective defenses of the immune system that help eradicate pathogens. The principal function of them is to interfere with viral multiplication (inhibition of viral transcription and translation). One of the interesting features of them is that they are host cell specific but not virus specific. Human interferons are classified into to 3 main principal types:

✓ Alpha-interferon (α-IFN) → produced by leucocytes

✓Beta-interferon (β-IFN) → produced by fibroblasts

✓ Gamma-interferon (γ-IFN) → produced by T-lymphocytes

IFNs also have other functions: they activate immune cells, such as natural killer cells and macrophages; they increase host defenses by up-regulating antigen presentation by virtue of increasing the expression of major histocompatibility complex (MHC) antigens. Certain symptoms of infections, such as fever, muscle pain and "flu-like symptoms", are also caused by the production of IFNs and other cytokines.

COMPLEMENT SYSTEM

The term "**complement**" was coined by Paul Ehrlich to describe the activity in serum, which could "complement" the ability of specific antibody to cause

lysis of bacteria. Complement historically refers to fresh serum capable of lysing antibody-coated cells.

Complement system is composed of more than 25 different proteins produced by hepatocytes, macrophages and intestinal epithelial cells. Fibroblasts and intestinal epithelial cells make C1, while the liver makes C3, C6, and C9. They are present in the circulation as inactive molecules. Though some components are resistant to heat, heating serum at 56 °C for 30 minutes destroys complement's activity. Serum complement levels, especially C3, often drop during infection as complement is activated faster than it is produced. Complement proteins can be quantified directly by ELISA.

Complement proteins work in a cascade, where the binding of one protein promotes the binding of the next protein in the cascade. Complement components are numbered in the order in which they were discovered. During activation, some complement components are split into two parts. The larger part of the molecule called "b" while the smaller fragment called "a" may diffuse away.

In most cases it is the "b" fragment binds to the surface of the cell to be lysed (the fragments of C2 are an exception to this rule: C2a binds to the membrane while C2b is freed into serum or tissue spaces). Inactivated fragments are indicated by a small "i". Enzymatically active forms are symbolized by a bar over the letter or number.

Activation of complement results in the production of several biologically active molecules, which contribute to non-specific immunity and inflammation. Complement is not antigen-specific and it is activated immediately in the presence of pathogen, so it is considered part of innate immunity.

The complement activation can be divided into three pathways, classical, lectin and alternative, all of which result in the activation of C5 and lead the formation of the membrane attack complex (MAC).

CLASSICAL PATHWAY

The classical pathway is triggered primarily by immune complexes (containing antigen and IgG or IgM) in the presence of complement components.

Direction of classic cascade activation: **C1** → **C4** (C4a and C4b) → **C2** (C2a and C2b) → **C3** (C3a and C3b) → **C5** (C5a and C5b) → **MAC**.

C1 is the first complement component to participate in classical pathway. It is composed of C1q, C1r and C1s. Binding of C1q to Ag-Ab complexes results in autocatalysis of C1r. The altered C1r cleaves C1s and this cleaved C1s is capable of cleaving both C4 and C2. Activated C1s enzymatically cleaves C4 into C4a and C4b. C4b binds to the Ag-bearing particle or cell membrane while C4a remains a biologically active peptide at the reaction site. C4b binds C2, which becomes susceptible to C1s and is cleaved into C2a and C2b. C2a remains complexed with C4b whereas C2b is released. C4b2a complex is known as **C3 convertase**. C3 convertase, in the presence of Mg^{2+} cleaves C3 into C3a and C3b. C3b binds to the membrane to form C4b2a3b complex whereas C3a remains in the microenvironment.

C4b2a3b complex functions as **C5 convertase**, which cleaves C5 into C5a and C5b. Generation of C5 convertase marks the end of the classical pathway. C5b initiates the formation of **membrane attack complex** (MAC).

ALTERNATIVE PATHWAY:

Alternate pathway is so called because it bypasses the requirement of antigen-antibody complex, C1, C2 and C4 components. It begins with the spontaneous activation of C3 in serum and requires Factors B and D, all present in normal serum. A C3b-like molecule (C3i) is generated by slow hydrolysis of native C3. C3i binds factor B, which is cleaved by Factor D to produce C3iBb. C3iBb cleaves native C3 into C3a and C3b. C3b binds factor B, which is again cleaved by Factor D to produce C3bBb (now **C3 convertase**). C3b has very short life and unless stabilized by membrane or molecule present on many pathogens, it is quickly inactivated. In the absence of such a molecule, it binds quickly to RBC.

Protein, properdin (factor P), further stabilizes this complex. Hence the alternative pathway is also known as the properdin pathway. Stabilized C3 convertase cleaves more C3 and produces C3bBb3b complex (**C5 convertase**), which cleaves C5 into C5a and C5b. C5b initiates the formation of MAC.

The alternative pathway provides a means of non-specific resistance against infection without the participation of antibodies and hence provides a first line of defense against a number of infectious agents. Some of the microbial components, which can activate the alternative complement cascade, include LPS from Gram-negative outer membranes (mainly Neisseria), teichoic acid from Gram-positive cell walls, certain viruses, parasites, zymosan from fungal and yeast cell walls and some parasite surface molecules.

FORMATION OF MAC:

C5 convertase, generated by any of the pathways described above, cleaves C5 into C5a and C5b. C5b instantaneously binds C6 and subsequently C7 to yield a hydrophobic C5b67 complex, which attaches quickly to the plasma membrane. Subsequently, C8 binds to this complex and causes the insertion of several C9 molecules. The insertion of membrane attack complex causes formation of a hole in the membrane thus lysing the cell. If complement is activated on an antigen without a lipid membrane to which the C567 can attach, the C567 complex may bind to nearby cells and initiate bystander lysis.

LECTIN PATHWAY:

C4 activation can be achieved without antibody and C1 participation via the lectin pathway (mannan-binding lectin/protein (MBL) initiates this pathway).

Functions of complement system

1. Increase rate of inflammation (C3a, C4a, C5a are called **anaphylotoxins**).
2. Opsonization of microbes (C3b, C5b are called **opsonins**).
3. Damage of pathogens (formation of **MAC** which causes microbial lysis).

Lecture 5. IMMUNE SYSTEM OF HUMAN BODY. IMMUNOLOGICAL METHODS OF DIAGNOSIS OF THE INFECTIOUS DISEASES

Immune system:

• System for protection against infecting and other foreign agents (antigens — Ag) by distinguishing self from non-self.

• Provide the immunity — CMI or AMI.

Functions:

1. Defense from "not-self".
2. Elimination of modified "self" (cancer cells).
3. Regulation of cells growth.
4. Regulation the homeostasis of human organism.

Functional organization of the immune system:

•Organic level.

•Cellular level.

•Molecular level.

ORGANIC LEVEL

1.**Primary (central) organs**:

•Thymus ►T-cells maturation (T-lymphopoiesis);

•Bone marrow ►All the immune cells are born and only B-cells mature (B-lymphopoiesis).

2. **Secondary (peripheral) organs**:

•Provide specific immune response (interaction of B- and T-cell with Ag).

•Lymph nodes and spleen.

•Mucous associated lymphoid tissue (MALT) including tonsils, Peyer's patches, follicles, appendix and others.

•Skin associated lymphoid tissue (SALT).

•Gut associated lymphoid tissue (GALT).

MUCOSA ASSOCIATED LYMPHOID TISSUE (MALT):

Approximately >50 % of lymphoid tissue in the body is found associated with the mucosal system. MALT is composed of gut-associated lymphoid tissues (GALT) lining the intestinal tract, bronchus-associated lymphoid tissue (BALT) lining the respiratory tract, and lymphoid tissue lining the genitourinary tract. The respiratory, alimentary and genitourinary tracts are guarded by subepithelial accumulations of lymphoid tissue that are not covered by connective tissue capsule. They may occur as diffuse collections of lymphocytes, plasma cells and phagocytes throughout the lung and *lamina propria* of intestine or as clearly organized tissue with well-formed lymphoid follicles. The well-formed follicles include the tonsils (lingual, palatine and pharyngeal), Peyer's patches in the intestine and appendix. The major function of these organs is to provide local immunity by way of sIgA (also IgE) production. Diffuse accumulations of lymphoid tissue are seen in the la-

mina propria of the intestinal wall. The intestinal epithelium overlying the Peyer's patches is specialized to allow the transport of antigens into the lymphoid tissue. This function is carried out by cuboidal absorptive epithelial cells termed "M" cells, so called because they have numerous microfolds on their luminal surface. M cells endocytose, transport and present antigens to subepithelial lymphoid cells. Majority of intra-epithelial lymphocytes are T-cells, and most often CD8+ lymphocytes. The intestinal lamina propria contains CD4+ lymphocytes, large number of B-cells, plasma cells, macrophages, dendritic cells, eosinophils and mast cells. Peyer's patches contain both B-cells and CD4+ T-cells.

CELLULAR LEVEL

1. **Antigen presenting cells** (APCs): macrophages, dendritic cells, B-cells.
2. **Regulatory cells**: T-helpers, T_{reg}, B_{reg}.
3. **Effector cells**: B-cells, CTLs **(C**ytotoxic **T-l**ymphocytes, outdated name is T-killers), neutrophils, macrophages, NK-cells, mast cells.
4. **Memory cells**: memory T- and B-cells.

ANTIGEN PRESENTING CELLS

An antigen-presenting cell (APC) or accessory cell is a cell that displays foreign antigens complexed with major histocompatibility complexes (MHCs) on their surfaces; this process is known as **antigen presentation**. T-cells may recognize these complexes using their TCR. These cells process antigens and present them to T-cells.

APCs fall into two categories: professional or non-professional.

T-cells cannot recognize, and therefore cannot respond to "free" antigen. T-cells can only "see" an antigen that has been processed and presented by cells via carrier molecules like MHC and CD1 molecules. Most cells in the body can present antigen to CD8+ T-cells via MHC class I molecules and, thus, act as "APCs"; however, the term is often limited to specialized cells that can prime T-cells (i.e., activate a T-cell that has not been exposed to antigen, termed a naive T-cell). These cells, in general, express MHC class II as well as MHC class I molecules, and can stimulate CD4+ ("helper") T-cells as well as CD8+ ("cytotoxic") T-cells, respectively.

To help distinguish between the two types of APCs, those that express MHC class II molecules are often called **professional antigen-presenting cells**. Professional APCs are very efficient at internalizing antigen, either by *phagocytosis* or by *receptor-mediated endocytosis*, and then displaying a fragment of the antigen, bound to a class II MHC molecule, on their membrane. The T-cell recognizes and interacts with the antigen-class II MHC molecule complex on the membrane of the antigen-presenting cell. An additional co-stimulatory signal is then produced by the antigen-presenting cell, leading to activation of the T-cell. The expression of co-stimulatory molecules is a defining feature of professional APCs.

There are three main types of *professional antigen-presenting cells*:

✓ **Dendritic cells** (DCs), which have the broadest range of antigen presentation, and are probably the most important APC. Activated DCs are especially

potent Th cell activators because, as part of their composition, they express co-stimulatory molecules such as B7. This B7 co-stimulator of mature interdigitating DCs interacts with surface CD28 of naïve T-cell.

Dendritic cells are derived from myeloid progenitor in the bone marrow and are morphologically identified by spiny membranous projection on their surfaces. Immature dendritic cells are located in epithelia of skin, gastrointestinal tract and respiratory tract and are called **Langerhans cells**. They express low levels of MHC proteins on their surface and their main function is to capture and transport protein antigen to the draining lymph node. During their migration to the lymph node, dendritic cells mature into excellent APC. Mature dendritic cells reside in the T-cell area (paracortex) of the lymph node. Here, they are referred as **interdigitating dendritic cells**. These cells are distinct from the dendritic cells that occur in the germinal centers of lymphoid follicles (**follicular dendritic cells**) in lymph node, spleen and MALT. The follicular dendritic cells are not derived from the bone marrow and their role is to present antigen-antibody complex and complement products to B-cell.

✓ **Macrophages**.

✓ **Certain B-cells**, which express B-cell receptor and secrete a specific antibody, can internalize the antigen, which bind to BCR and present it to MHC II (but B-cells are inefficient APC for most of antigens).

Nonprofessional APC do not constitutively express MHC class II proteins required for interaction with naive T-cells; these are expressed only upon stimulation of the non-professional APC by certain cytokines such as IFN-γ. Non-professional APCs include: fibroblasts (skin), some epithelial cells, glial cells (brain), pancreatic beta cells, vascular endothelial cells.

After APCs have phagocytosed pathogens, they usually migrate to the vast networks of lymph vessels and are carried via lymph flow to the draining lymph nodes. The lymph nodes become a collection point to which APCs such as DCs can interact with T-cells. They do this by chemotaxis, which involves interacting with chemokines that are expressed on the surface of cells (e.g., endothelial cells of the high endothelial venules) or have been released as chemical messengers to draw the APCs to the lymph nodes. During the migration, DCs undergo a process of maturation; in essence, they lose most of their ability to further engulf pathogens, and they develop an increased ability to communicate with T-cells. Enzymes within the cell digest the swallowed pathogen into smaller pieces containing epitopes, which are then presented to T-cells using MHC.

IMMUNOCOMPETENT CELLS

Immunocompetent cells are cells which participate in the development of immune response (T- and B-cells).

B-cells

B-cells make antibodies against antigens, to perform the role of antigen-presenting cells (APCs), and to develop into memory B-cells after activation by antigen interaction.

B-cell development occurs through several stages, each stage representing a change in the genome content at the antibody loci. Ab is composed of two identical light (L) and two identical heavy (H) chains, and the genes specifying them are found in the 'V' (Variable) region and the 'C' (Constant) region. In the H-chain 'V' region there are three segments; V, D, and J, which recombine randomly, in a process called **VDJ recombination**, to produce a unique variable domain in the Ig of each individual B-cell. Similar rearrangements occur for L-chain 'V' region except there are only two segments involved: V and J.

When the B-cell fails in any step of the maturation process, it will die by a mechanism called apoptosis, here called **clonal deletion**. B-cells are continuously produced in the bone marrow, upon leaving of bone marrow B-cells are carried out to the lymph nodes, Peyer's patches, tonsils and other peripheral immune organs The human body makes millions of different types of B-cells each day that circulate in the blood and lymphatic system performing the role of immune surveillance. They do not produce antibodies until they become fully activated. Each B-cell has a unique receptor protein (B-cell receptor − BCR) on its surface that will bind to one particular antigen. Once a B-cell encounters its cognate antigen and receives an additional signal from a T helper cell, it can further differentiate into one of the two types of B-cells: plasma B-cells and memory B-cells. The B-cell may either become one of these cell types directly or undergo an intermediate differentiation step, the germinal center reaction, where the B-cell will *hypermutate* the variable region of its immunoglobulin gene ("**somatic hypermutation**") and possibly undergo **class switching**.

Table 6 — Characteristics that distinguish T- from B-lymphocytes

Characteristic	T-cells	B-cells
Cell type	Mononuclear leukocyte (lymphocyte)	Mononuclear leukocyte (lymphocyte)
Ag binding receptor	**T-cell receptor** with co-receptor (TCR/CD3) TCR recognize protein Ag with help of MHC	**B-cell receptor** with co-receptor (BCR/CD79a/b) BCR can recognize soluble Ag of any chemical composition (without MHC help)
Surface markers	CD3, CD4, CD4	CD 19, CD20, CD21, CD40
Chief secretary products	Lymphokines	Antibodies

B-cell types

- **Plasma B-cells** (plasmocytes or effector B-cells) are large B-cells that have been exposed to antigen and produce and secrete large amounts of antibodies. An electron micrograph of these cells reveals large amounts of rough endoplasmic reticulum, responsible for synthesizing the antibody, in the cell's cytoplasm. These are short-lived cells and undergo apoptosis when the inciting agent that induced immune response is eliminated.
- **Memory B-cells** differentiate from activated B-cells predominantly from a germinal center and are specific to the antigen encountered during the primary

immune response. These cells are able to live for a long time, and can respond quickly following a second exposure to the same antigen.

• **B-1 cells** express IgM in greater quantities than IgG and their receptors show *polyspecificity*, meaning that they have low affinities for many different antigens. Polyspecific immunoglobulins often have a preference for other immunoglobulins, self-antigens, and common bacterial polysaccharides. B-1 cells are present in low numbers in the lymph nodes and spleen and are instead found predominantly in the peritoneal and pleural cavities.

• **B-2 cells** are the cells intended when using the unqualified "B-cell".

• **Regulatory B-cells** (B_{regs}) are B-cells involved in immune regulation via various mechanisms including secretion of IL-10. Subsets of B_{regs} are found both within the B-1 and B-2 cell population.

T-cells types

T-cells are a type of lymphocyte that plays a central role in immune response. The several subsets of T-cells each have a distinct function.

• **T helper cells** assist in maturation of B-cells into plasma cells and memory B-cells, and activation of cytotoxic T-cells and macrophages. These cells are also known as **CD4+ T cells** because they express the CD4 glycoprotein on their surfaces. Helper T cells become activated when they are presented with peptide antigens by MHC class II molecules, which are expressed on the surface of antigen-presenting cells (APCs). Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response. These cells can differentiate into one of several subtypes, including Th1, Th2, Th3, Th17, follicular helper T-cells or T_{FH}, which secrete different cytokines to facilitate different types of immune responses. Signalling from the APC directs T-cells into particular subtypes.

• **Th1** produce IL–2, gamma IFN and lymphotoxin (TNF) → activation of macrophages, NK, neutrophils, CTLs (→ provide CMI).

• **Th2** produce IL–2, 4, 5, 6, 10, 13 → activation of B-cells, mast cells and eosinophils (→ provide AMI).

• **Cytotoxic T-cells** (CTLs) destroy virus-infected cells and tumor cells, and are also implicated in transplant rejection. These cells are also known as **CD8+ T-cells** since they express the CD8 at their surfaces. These cells recognize their targets by binding to antigen associated with MHC class I molecules, which are present on the surface of all nucleated cells.

• Memory T-cells are a subset of antigen-specific T-cells that persist long-term after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen, thus providing the immune system with "memory" against past infections. Memory T-cells typically express the cell surface protein CD45RO.

• **Regulatory T-cells** (T_{reg} cells), formerly known as suppressor T-cells, are crucial for the maintenance of immunological tolerance. Their major role is to shut down

T cell-mediated immunity toward the end of an immune reaction and to suppress autoreactive T-cells that escaped the process of negative selection in the thymus.

Regulatory T-cells can develop either during normal development in the thymus, and are then known as **thymic T_{reg} cells**, or can be induced peripherally and are called **peripherally derived T_{reg} cells**. These two subsets were previously called "naturally occurring", and "adaptive" or "induced", respectively.

• **Gamma delta T-cells** (γδ T-cells) represent a small subset of T-cells that possess a distinct TCR on their surfaces (one γ-chain and one δ-chain). This group of T-cells is found in the highest abundance in the gut mucosa, within a population of lymphocytes known as *intraepithelial* lymphocytes. The antigenic molecules that activate γδ T-cells are still widely unknown. However, γδ T-cells are not MHC-restricted and seem to be able to recognize whole proteins rather than requiring peptides to be presented by MHC molecules on APCs.

MOLECULAR LEVEL (**Ig super family**):

(1) **BCR** – **B**-**c**ell **r**eceptor for Ag recognition (structure of BCR: membrane Ig M and Ig D and co-receptor CD79 a/b).

(2) **TCR** – **T**-**c**ell **r**eceptor for Ag recognization (structure of TCR: alpha+beta or delta+gamma globulin chains with constant (C) and variable (V) regions and co-receptor CD3).

A critical difference between B-cells and T-cells is how each lymphocyte recognizes its antigen. B-cells recognize their cognate antigen in its native form. They recognize free (soluble) antigen in the blood or lymph using their BCR. In contrast, T-cells recognize their cognate antigen in a processed form, as a peptide fragment presented by MHC molecule to TCR.

(3) **Soluble immunoglobulins** (IgG, IgM, IgD, IgA, IgE).

(4) **Antigen-presenting molecules**: human leukocyte antigens (HLA) or major histocompatibility complex (MHC).

(5) **Cytokines.**

(6) **Adhesins** (ICAM-1 or **I**nter**c**ellular **a**dhesion **m**olecule 1 which is generally expressed on endothelium and immune cells).

(7) **CD** molecules ("cluster of differentiation" or cluster designation markers) that are expressed on the cell membranes and provide the information about specify of the cells, their origin, stage of activation and differentiation. Each marker has been given CD number such as CD1, CD2, CD3, etc. For example, it is known that HIV infects and destroys the CD4+ T-cells, also known as T-helpers.

Cytokines

Cytokines are a broad and loose category of small proteins that are important in cell signaling. They are released by cells and affect the functions of other cells. Cytokines are produced by a broad range of cells, including immune cells like macrophages, B-lymphocytes, T-lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells.

They act through receptors, and are especially important in the immune system; cytokines modulate the balance between CMI and AMI; regulate the maturation, growth, and responsiveness of particular cell populations.

They are different from hormones, which are also important cell signaling molecules, in that hormones circulate in much lower concentrations and hormones tend to be made by specific kinds of cells.

They are important in health and disease, specifically in host responses to infection, immune responses, inflammation, trauma, sepsis, cancer, and reproduction.

Types of cytokines:

• **Interleukins** (IL) was initially used by researchers for those cytokines whose presumed targets are principally leukocytes. It is now recognized that the vast majority of these are produced by T-helper cells and provide all kinds of regulations in the immune system.

• **Colony-stimulating factor** (CSF) → support the growth of cells.

• **Interferon** (IFN) → involved in antiviral response.

• **Tumor necrosis factor** (TNF) → can cause cell death (apoptosis).

• **Chemokines** → mediate chemoattraction (chemotaxis) between cells.

• **Monokines** → produced exclusively by monocytes.

• **Lymphokines** → produced by lymphocytes.

IMMUNOLOGICAL METHOD OF DIAGNOSIS

Immunological (serological) method is a method based on the specific interaction between antigens and antibodies.

Aims of the serological method:

(1) **Serological diagnosis** (**serodiagnostics**) of the infection disease is based on detection of unknown Ab in serum of patient with help of known specific Ag in **diagnostics**.

Principles of the giving during serodiagnostics:

• Comparison of patient's Ab titer with *diagnostic titer* for the certain disease.

• *Increase titer* in the paired sera of the patient in ≥ 4 times.

• *Detection of classes of Ig with help of ELISA*: IgM is sign for the acute infection and IgG is sign for the chronic infection or immunity.

(2) Serological diagnosis of the infection diseases which is based on detection of unknown microbial Ag in the biological liquids or tissues of the patient with help of known Ab in diagnostic antisera is called **express-diagnostics**.

(3) Serological identification of the unknown Ag of pure culture (serotype of the pure culture) with help of known Ab in diagnostic antisera is called **serotyping.**

Diagnosticum is known killed microbes or Ag (O-diagnosticum, H-diagnosticum, erythrocytic diagnosticum, viral diagnosticum, etc.)

Diagnostic antiserum is a serum containing specific Ab of known specificity and titer (monovalent serum, polyvalent serum, agglutinating serum, precipitating serum, antitoxic serum, antiglobulin serum, etc.).

Serological method includes serological reactions (agglutination, precipitation, neutralization, IFA, ELISA, etc.).

SEROLOGICAL REACTIONS are reactions between Ag and Ab on the basis their specificity to each other and formation of immune complexes (IC).

DOUBLE SEROLOGICAL REACTIONS

❖ **REACTION OF AGGLUTINATION** (RA):

• Sticking of particulate Ag (agglutinogen: bacteria, erythrocytes) with specific Ab (agglutinins) in the presence of an electrolyte.

• Ag and Ab react must be specific to each other.

• Positive result → formation of immune complex — agglutinate ("small white grains" in the transparent drop of a serum).

• RA classification: **direct** (may be slide or tube agglutination) and **indirect/passive** (may be latex-agglutination — RLA, co-agglutination — RCA and indirect hemagglutination – RIHA).

❖ **REACTION OF PRECIPITATION** (RP):

• Sedimentation of soluble Ag (precipitinogen: exotoxin, drug) with specific Ab (precipitinins) in presence of an electrolyte.

• Ag and Ab react must be specific to each other.

• Positive result → formation of insoluble immune complexes — precipitate (appearance of turbidity in liquid or lines/bands of precipitation in gel.

• RP classification: in liquid phase — tube (ring) precipitation, flocculation; in gel – immunodiffusion, immunoelectrophoresis.

Precipitation in a gel is called **immunodiffusion.**

Types of immunodiffusion:

1. Radial immunodiffusion.
2. Single immunodiffusion.
3. Double immunodiffusion (Ouchterlony test).

❖ **REACTION NEUTRALIZATION** (RN) is based on neutralization of effects of exotoxins or viruses by specific antibodies.

Types of neutralization in vivo:

1. Skin tests for estimation antitoxic immunity (Dick test, Shick test):

2.RN of toxins or viruses on animals.

Also flocculation and **Elec test** for *C.diphtheriae* can be considered as neutralization tests (RP+RN).

COMPLEX SEROLOGICAL REACTIONS

COMPLEMENT FIXATION TEST (CFT):

• Ability of Ag-Ab complex to "fix" complement.

• Indicator system is **hemolytic system** (consists of erythrocytes of sheep and hemolytic serum).

• Source of a complement is guinea pig serum or dry complement.

• Positive CFT is indicated by the negative hemolysis, negative CFT is indicated by the positive hemolysis.

LABELED REACTIONS

❖ **IMMUNOFLUORESCENCE REACTION** (IFA):

• Principle: "labeling" (marking) of Ab or anti-Ab with fluorescent dye which converts ultraviolet rays into visible light (use luminescent microscopy for detection of the results).

• Positive IFA: green shining of microbes (Ag) on the dark background.

• IFA may be direct and indirect.

❖ **ENZYME LINKED IMMUNOSORBENT ASSAY** (ELISA):

• Principle: "labeling" (marking) of Ab or anti-Ab with enzyme (peroxidase) which decompose substrate and it changes color due to chromogen (use photometry for detection of the results).

• Positive ELISA: colorless substrate becomes substrate of yellow color.

• Known Ag or Ab are adsorbed on the solid phase.

• ELISA may be direct and indirect.

❖ **IMMUNOBLOTTING** (Western blot):

• Principle: electrophoresis + ELISA.

• Whole microbes are decomposed into certain Ag with help of electric force in gel according to their molecular mass (electrophoresis.

• Then these Ag are adsorbed on the cellulose paper ("blots") and other steps of reaction are carried out as ELISA.

• Positive result: appearance dark bands on the cellulose paper.

❖ **RADIOIMMUNE ASSAY** (RIA):

Principle: "labeling" of Ab or Ag with **radioactive isotope** and detection of the results with help of radioactive counters.

Lecture 6. ANTIGENS. IMMUNE RESPONSE. CELL-MEDIATED IMMUNE RESPONSE (CMI)

Antigen is certain kind of substances foreign to the host and able to trigger the immune response in the host (formation of Ab and binding with them, activation of immune competent cells and binding with their receptors).

Antigens are large molecules which have specific chemical groups — **antigenic determinants**, or **epitopes** (will react with paratopes of antibodies or TCR/BCR of lymphocytes).

Attributes of antigenicity:

• **IMMUNOLOGICAL REACTIVITY** is specific reaction of Ag with Ab or the immune cells.

• **IMMUNOGENICITY** is an ability of Ag to induce the immune response.

Types of Ag:

1. *Complete Ag* or immunogens (induce Ab formation and can bind with Ab so produced). Ex.: bacterial and viral Ag.

2. *Haptens* or incomplete Ag (incapable to induce Ab formation but can react specifically with Ab). They become immunogenic after combining with a protein molecule — carrier. Ex.: low-molecular weight compounds like antibiotics.

Classification of Ag:

• **On origin:** exogenic and endogenic Ag.

• **On composition:** protein and non-proteins.

• **On participation of T-helpers** in activation of B-cells: T-dependent and T-independent Ag.

• **On immunogenicity:** haptens and immunogens.

• **On number of epitopes**: monovalent and polyvalent.

Determinants of antigenicity:

Size: the most potent antigens are usually large proteins. Generally, molecules with a molecular weight less than 5 000 are weakly immunogenic, and very small ones (aminoacids) are nonimmunogenic. Certain small molecules (haptens) become immunogenic only when linked to a carrier protein.

Chemical nature: most naturally occurring antigens are proteins and polysaccharides. Lipids and nucleic acids are less antigenic. Their antigenicity is enhanced by combination with proteins. A certain degree of structural diversity is required for antigenicity. However, not all proteins are antigenic. A well-known exception is gelatin, which is nonimmunogenic because of its structural unstability.

Susceptibility to tissue enzymes: Only substances which are metabolized and are susceptible to the action of tissue enzymes behave as antigens. Phagocytosis and intracellular enzymes appear to play an essential role in breaking down antigens into immunogenic fragments. Substances unsusceptible to tissue enzymes are not antigenic. Substances very rapidly broken down by tissue en-

zymes are also not antigenic.

Foreignness: only antigens which are "foreign" to the individual (*nonself*) induce an immune response. The animal body contains numerous antigens which induce an immune response when introduced into another individual or species. Molecules recognized as "self" are not immunogenic. Tolerance of self-antigens is conditioned by contact with them during the development of the immune apparatus. Breakdown of this homeostatic mechanism results in autoimmunization and autoimmune disease.

Antigenic specificity:

- **Species specificity**: tissues of all individuals in a species contain species specific antigens. There exists some degree of cross reaction between antigens from related species. Phylogenetic relationships are reflected in the extent of cross reaction between antigens from different species that cause hypersensitivity.
- **Organ and tissue specificity**: some organs, such as the brain, kidney and lens protein of different species, share the same antigen. Such antigens, characteristic of an organ or tissue and found in different species, are called organ specific antigens.
- **Isospecificity:** isoantigens are antigens found in some but not all members of a species. A species may be grouped depending on the presence of different isoantigens in its members. The best examples of isoantigens are the human erythrocyte antigens based on which individuals can be classified into different blood groups. These are genetically determined. They are of clinical importance in blood transfusion and in isoimmunisation during pregnancy. Histocompatibility antigens (MHC) are those cellular determinants specific to each individual of a species. They are recognized by genetically different individuals of the same species when attempts are made to transfer or transplant cellular material from one individual to another.
- **Autospecificity**: individual may respond immunologically against his or her own body constituents (it is autoantigens), with the potential of producing of tissue damage (autoimmune diseases).
- **Heterogenetic specificity**: the same or closely related antigens may sometimes occur in different biological species, classes and kingdoms. These are known as heterogenetic antigens, best exemplified by the Forssmann antigen which is a lipid carbohydrate complex widely distributed in many animals, birds, plants and bacteria. It is absent in rabbits, so anti-Forssman antibody can be prepared in these animals.

Bacterial Ag:

- Group Ag (for some species).
- Specific Ag (for species).
- Typical Ag (for certain serotype).
- Capsular Ag (K-Ag).
- Cell wall Ag (O-Ag).
- Flagella Ag (H-Ag).
- Ribosomal Ag.

• Ag of toxins, enzymes.

Protective Ag (highly immunogenic and provide development of the protective level of Ab in immunity).

SYPERANTIGENS are Ag which induce non-specific multiple activation of T-lymphocytes and after activation lead to its apoptosis. Ex.: enterotoxins, cholerotoxin, staphylococcal exotoxins, some viruses.

Pathogen-associated molecular patterns (PAMP) are molecules associated with groups of pathogens that are recognized by cells of the innate immune system. These molecules can be referred to as small molecular motifs conserved within a class of microbes. They are recognized by ***Toll-like receptors*** (TLRs) and other ***pattern recognition receptors*** (PRRs).

They activate innate immune responses, protecting the host from infection, by identifying some conserved non-self molecules. LPS is considered to be the prototypical PAMP. LPS is specifically recognized by TLR 4 (recognition receptor of the innate immune system). Other PAMPs include bacterial flagellin, lipoteichoic acid from Gram-positive bacteria, peptidoglycan, and nucleic acid variants normally associated with viruses (dsRNA), etc.

Damage-associated molecular pattern molecules (DAMPs) also known as danger-associated molecular pattern molecules are molecules that can initiate and perpetuate immune response in the noninfectious inflammatory response (in contrast PAMPs initiate and perpetuate the infectious pathogen inflammatory response). Many DAMPs are nuclear or cytosolic proteins which released outside the cell or exposed on the surface of the cell following tissue injury or necrosis.

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

MHC is collection of highly polymorphic genes encoding the proteins which regulate immune response. **Antigens of MHC** are surface glycoproteins of the different cells which responsible for **immune recognization** of CD receptors during Ag presentation for T-cells (they help T-cells to recognize and bind Ag). May be MHC class I and MHC class II (also MHC class III that encode complement molecules and cytokines). In humans, MHC is called **HLA** (their corresponding genes are found on the short arm of chromosome 6).

Table 11 — Characteristic features of different MHC classes

Feature	MHC I	MHC II
Localization	All nucleated cells and platelets	Antigen-presenting cells (macrophages, B-cells, dendritic cells)
Types	HLA-A, HLA-B, HLA-C	HLA-DP, HLA-DQ, HLA-DR
Function	Ag recognition by T killers (bind with CD8)	Ag recognition by T helpers (bind with CD4)
Structure	Alpha heavy chain (3 domains — α1, α2, α3, light β2-microglobulin)	Alpha (α1 and α2) and beta chains (β1 and β1)

Importance of MHC:

• Immunological recognization of Ag by the immune competent cells for

development of immune response (antigen presentation).

• Immunogenetical and biological individuality of every organism (marker of "self" on every cell).

• Importance in compatibility of transplanted tissues.

ANTIGEN PRESENTATION

❖ **Presentation of Ag for activation T helpers**:

Base of presentation is interaction between APC (dendritic cell, macrophage) and T-helpers.

► Ag is recognized by TCR of T-helper.

► MHC II class is recognized by CD4 receptor of T-helper.

❖ **Presentation of Ag for activation of CTLs**:

Base of presentation is interaction between CTL and target cells.

► Ag is recognized by TCR of CTL.

► MHC I class is recognized by CD8 of CTL.

IMMUNE RESPONSE

Immune response is specific reactivity induced in a host by antigen. In infectious disease is known it is generally equated with protection against pathogen ("immunity"). But the immune response has a much wider scope and includes reactions against any antigen, living (bacteria, fungi) or nonliving (pollen, food). Result of immune response may be beneficial, indifferent or injurious to the host. The immune response can be two types — AMI and CMI. The two are usually developed together, though at times one or the other may be predominant or exclusive. They usually act in connection but may sometimes act in opposition.

AMI provides:

• Defense against most of the pathogens with predominant extracellular localization (bacteria).

• Primary defense against viruses that infect respiratory or intestinal tracts.

• Participation in the pathogenesis of immediate type of hypersensitivity and certain autoimmune diseases.

• Formation of antitoxic immunity (against exotoxins of bacteria).

CMI provides:

• Protection against fungi, protozoa and most of viruses.

• Protection against obligate and facultative intracellular bacterial pathogens.

• Participation in rejection of transplanted tissues and organs.

• Participation in the pathogenesis of delayed type of hypersensitivity and certain autoimmune diseases.

• Formation of antitumoral immunity.

Mechanisms of avoiding of immune response by pathogens

➢ Bacteria often overcome physical barriers by secreting enzymes that digest the barrier, for example, by using a type II secretion system. Alternatively, using a type III secretion system, they may insert a hollow tube into the host

cell, providing a direct route for proteins to move from the pathogen to the host.

➢ An evasion strategy used by several pathogens to avoid the innate immune system is to hide within the cells of their host (also called intracellular pathogenesis). Here, a pathogen spends most of its life-cycle inside host cells, where it is shielded from direct contact with immune cells, antibodies and complement. Some examples of intracellular pathogens include viruses, the food poisoning bacterium Salmonella and the eukaryotic parasites that cause malaria (*Plasmodium falciparum*) and leishmaniasis (Leishmania spp.).

➢ One role of *capsules* in bacterial virulence is to protect the bacteria from complement activation and phagocytosis. Polysaccharide capsules can hide bacterial components such as LPS or peptidoglycan which can induce the alternate complement pathway.

➢ Many pathogens secrete compounds that diminish or misdirect the host's immune response. This means that the host shows depressed immune responses to antigens in general, including those of the infecting pathogen (chronic bacterial infections such as leprosy and tuberculosis).

➢ Some bacteria form biofilms to protect themselves from the cells and proteins of the immune system.

➢ Other bacteria generate surface proteins that bind to antibodies, rendering them ineffective; examples include Streptococcus (protein M), *Staphylococcus aureus* (protein A). Some pathogens produce enzymes that destroy antibodies (IgA proteases that inactivate secretory IgA).

➢ The mechanisms used to evade the adaptive immune system are more complicated. The simplest approach is to rapidly change nonessential epitopes on the surface of the pathogen, while keeping essential epitopes concealed. This is called *antigenic variation*. An example is HIV, which mutates rapidly, so the proteins on its viral envelope that are essential for entry into its host target cell are constantly changing.

➢ *Molecular mimicry*. If a bacterial Ag is very similar to normal host "antigens", the immune responses to this Ag may be weak giving a degree of tolerance. In this case the antigenic determinants of the bacterium are so closely related chemically to host "self" components that the immunological cells cannot distinguish between the two and an immune response cannot be raised. Some bacterial capsules are composed of polysaccharides (hyaluronic acid, sialic acid) so similar to host tissue polysaccharides that they are not immunogenic.

CELL-MEDIATED IMMUNE RESPONSE (CMI)

Type of immunity that is mediated by specific effector T-cells and other different cytotoxic cells (macrophages, NK cells, etc).

Cells of CMI:

1. **APCs:** macrophages and dendritic cells.
2. **Effector T-cells**:

• T_{DTH} ► delayed type of hypersensitivity.

• CTLs ► specific extracellular cytotoxicity.

• NK cells ► natural extracellular cytotoxicity.

• K-cells ► ADCC (antibody dependent cell-mediated cytotoxicity).

• Macrophages ► intracellular nonspecific cytotoxicity (phagocytosis).

3. **Regulatory T-cells:** T-helpers (Th-1 is responsible for induction CMI, they produce cytokines: IL-2, gamma-IFN and TNF → activation of macrophages, neutrophils, CTLs); regulatory T-cells.

4. **Memory T-cells** → secondary immune response.

Main stages of CMI:

For example, during *viral infection*, viral epitopes are presented on the surface of the virus infected cell bound to MHC class I.

1. In this form viral epitopes are recognized by **cytotoxic T-cells** (CTLs). Mechanism of killing: CTLs during contact with target cell release the cytolytic molecules (*perforins* and *granzymes* → target cell apoptosis).

2. In order for expansion of the particular clone of cytotoxic T-cells to occur, **T-helpers** must also be activated. This occurs by phagocytosis of the virus by macrophages. Then viral epitopes are presented on the cell surface of macrophage together with MHC class II. T helpers recognize viral epitopes together with MHC class II and activate.

3. Activated T helpers (Th1) produce cytokines (IL–2, gamma-IFN, TNF) that stimulate differentiation and proliferation of both T-helpers themselves and CTLs.

4. Additionally **different cytotoxic cells** (macrophages, NK cells, K-cells) are activating and realizing of their cytotoxicity on the target cells.

5. $\mathbf{T_{DTH}}$ (type of Th) participate in pathogenesis of DTH (if antigen is allergen for immune system).

6. **T memory cells** are also forming; they remain fallowing an infection and are ready to develop immune response more rapidly.

Lecture 7. IMMUNOGLOBULINS. ANTIBODY-MEDIATED IMMUNE RESPONSE (AMI)

ANTIBODIES (IMMUNOGLOBULINS)

Antibodies belong to group of proteins known as **globulins.** Since these globulins are involved in the immune response, they are known as **immunoglobulins (Ig).**

Antibodies are proteins produced in response to Ag. They constitute 20–25 % of the total serum proteins. There are five classes of Ig have been recognized — IgG, IgA, IgE, IgM and IgD.

Clonal selection theory

Although many millions of B cells are present in the body, only a small number are capable to respond to any one antigen! Clonal selection theory explains how it works. When Ab is first produced by B cells, they are not secreted but are inserted into the cell membrane of B cell. When Ag comes into contact with B cell that has the corresponding Ab that B cell is triggered to divide and mature into a *clone of plasma cells, all produced Ab with the same specificity.* This clonal expansion accounts for increase in the number of the particular type of plasma cells.

Basic structure of Ig molecule

- The molecule is Y-shaped and consists of two heavy chains (**H-chain**) and two light chains (**L-chain**).
- Each of chain, in turn, consists of **variable region** and **constant region**. Constant regions for all molecules of the same Ig have the same amino acids. On other hand, variable region is the section of the molecule that interacts with the antigenic epitopes. Since each Ag is different from another, the variable regions of Ab are also different in their arrangement of amino acids.
- In the presence of papain (enzyme) Ig is spited into three fragments -one insoluble fragment which **c**rystallized in the cold (called **Fc-fragment**), and two identical soluble fractions which can bind with antigens (called **f**ragment **a**ntigen **b**inding — **Fab**).
- H-chains are distinct for each class and are designated by the Greek letter (IgG — γ/gamma/ H chain, IgA — α/alpha/, IgM — μ/mu/, IgE — ε/epsilon/, IgD — δ/delta/). L-chains are similar in all classes of Ig. Ig can have either κ/kappa/ or λ/lambda/, but never both.

CHARACTERISTICS OF EACH CLASS OF IMMUNOGLOBULINS

IgG

- Monomer (bivalent).
- 75–80 % in serum (8–16 mg/ml).
- Activation of complement.
- Neutralization of toxins and viruses.
- Opsonization during a phagocytosis.
- Transport across the placenta → passive immunity of newborn.

• ADCC (bind with K-cells).
• Late Ab (indicates about chronic infection or immunity after infection).

IgA

• 10–15 % in serum (0,6–4 mg/ml).
• Two forms:
1. Serum IgA (monomer).
2. Secretary IgA/sIgA (dimer or trimer) presents in secretions (tears, saliva, breast milk) and mucous and provide local immunity against respiratory and intestinal pathogens.
• Complement activation.

IgM

• 8–10 % in serum (0,5–2 mg/ml).
• Pentamer (valence 10).
• First Ig synthesized by fetus.
• First Ig which appears after Ag contact (indicates about acute infection).
• Activation of complement.
• Component of BCR (with IgD).

IgE

• Monomer (monovalent).
• Less than 0,003–0,5 % (few mg/ml).
• Ability to bind with mast cells → immediate type of hypersensitivity.
• High level indicates about helminthic infection.

IgD

• Monomer.
• Less than 0,3–0,5 %.
• Component of BCR (with IgM).

Generally about functions of Ab:

• IgG: protection body fluids (►secondary immune response).
• IgM: protection of bloodstream (►primary immune response).
• sIgA: protection body surfaces and mucous membranes (►local immunity).
• IgE: immediate type of hypersensitivity.
• IgD: BCR (recognition of Ag).

Table 12 — Classification of immunoglobulins

Property	Ig G	Ig A	Ig M	Ig E	Ig D
Serum concentration (mg/ml)	0,5–12	0,5–3	1,5	0,003–0,00003	0,03
Percentage of total immunoglobulins in serum	80	13	6	Less than 1	Less than 1
Half life (days)	23	6	5	1–5	2–8
Molecule weight	150,000	160,000	900,000	190,000	180,000
Complement fixation	Yes	No	Yes	No	No
Placental transport	Yes	No	No	No	No
Present in milk	Yes	Yes	No	No	No
Secretion by seromucous glands	No	Yes	No	No	NO

Number of subunits	Mono-mer	Dimer	Pentamer	Monomer	Mono-mer

Classification of Ab (Ig)

• Natural Ab (present in blood serum without activation by Ag).
• Immune Ab (accumulate in a serum after introduction of Ag).
• Circulating molecules: all serum Ig and sIgA.
• Components of BCR (IgM and IgD).
• Complete Ab → all Fab-components work best and can cause visible by eyes reaction of an agglutination.
• Incomplete Ab → can't cause visible reaction of agglutination.

Structural differences between immunoglobulins

Isotype is determined by the primary sequence of amino acids in the constant region of the heavy chain. For example, the five human isotypes, IgA, IgD, IgG, IgE and IgM are found in all humans and injection of human IgG into another human would not generate antibodies directed against Ig G isotype. Another means of classifying immunoglobulins is defined by the term **allotype**. Unlike isotypes, allotypes reflect genetic differences between members of the same species. Therefore, injection of any specific human allotype into another human could possibly generate antibodies directed against particular allotypic variation. A third means of classifying immunoglobulins is defined by the term **idiotype** that reflects the antigen binding specificity of any particular antibody molecule. Idiotypes are so unique that an individual person is probably capable of generating antibodies directed against their own idiotypic determinants.

Functions of antibodies in host defense

1. Opsonization: phagocytosis is greatly enhanced when it is coated by antibodies.
2. Steric hindrance: antibodies combine with the surfaces of microorganisms and may prevent their attachment to susceptible cells or mucosal surfaces.
3. Toxin neutralization: Toxin-neutralizing antibodies (antitoxins) react with a soluble bacterial toxin and block the interaction of the toxin with its specific target cell or substrate.
4. Agglutination and precipitation: antibodies combine with microorganisms and cause them to agglutinate or precipitate. This reduces the number of separate infectious units and makes them more readily phagocytosed because the clump of particles is larger in size.
5. Complement fixation: activation of the classic pathway occurs as a result of complement components binding to the CH2 region of Ig.
6. Allergy and anaphylaxis: Ag-specific Ig E may bind to the receptors on mast cells and promote their degranulation leading to the signs and symptoms of allergy.
7. Ab-dependent cell-mediated cytotoxicity (ADCC): antibodies stimulated by virus infection bind to the viral Ag expressed on the surface of the infected cells. The Fc-portion of Ig binds to the Fc-receptor bearing cells which are able to lyse the viral infected cells.
8. Effect on microbial physiology: some antibodies inhibit the movement organ-isms by attaching to flagella. Some Ab inhibits the metabolism of microbes.

Affinity and avidity

Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody. It is the sum of the attractive and repulsive forces operating between the antigenic determinant and the combining site of the antibody.

Affinity is the equilibrium constant that describes the antigen-antibody reaction. Most antibodies have a high affinity for their antigens. Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies. Avidity is influenced by both the valence of the antibody and the valence of the antigen. Avidity is more than the sum of the individual affinities.

Specificity and cross reactivity

Specificity refers to the ability of an individual antibody combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one antigen. In general, there is a high degree of specificity in antigen-antibody reactions. Antibodies can distinguish differences in 1) the primary structure of an antigen, 2) isomeric forms of an antigen, and 3) secondary and tertiary structure of an antigen.

Cross reactivity refers to the ability of an individual antibody combining site to react with more than one antigenic determinant or the ability of a population of antibody molecules to react with more than one antigen.

ANTIBODY-MEDIATED IMMUNE RESPONSE (AMI)

Type of immunity that is mediated by soluble host proteins called antibodies or immunoglobulins (it is also called **humoral immunity**).

There are two types of antigens which can induce AMI: **T-dependent antigens** (complex Ag like proteins and erythrocytes) and **T-independent antigens** (polysaccharides and other structurally simple molecules with repeating epitopes).

T-dependent activation of B-cells:

- Participation of T-helpers.
- All Ig classes are produced.
- B memory cells are present.

Once a pathogen is ingested by APC as a macrophage or dendritic cell, the pathogen's proteins are then digested to peptides and attached to a class II MHC protein. This complex is then moved to the outside of the cell membrane. The macrophage is now activated to deliver multiple signals to a specific T-cell that recognizes the peptide presented. The T-cell is then stimulated to produce cytokines, resulting in the proliferation or differentiation to effector or memory T-cells. A certain portion of the resulting effector T-cells then activates specific B-cells through a phenomenon known as an immunological synapse. Activated B-cells subsequently produce antibodies that assist in inhibiting pathogens until phagocytes (i.e., macrophages, neutrophils) or the complement system, for example, clear the host of the pathogen(s).

Most antigens are T-dependent, meaning T-cell help is required for maximal antibody production. With a T-dependent antigen, the first signal comes from antigen's cross-linking BCR, and the second signal comes from co-stimulation

provided by a T-cell. T-dependent antigens consist of peptides mounted on B-cell Class II MHC to be presented to T-cells (follicular helper T-cells or Th2 cell). When a B-cell processes and presents the same antigen to the primed Th cell, the T-cell secretes cytokines that activate the B-cell. These cytokines trigger B-cell proliferation and differentiation into plasma cells. Isotype switching to IgG, IgA, IgE and memory cell generation occur in response to T-dependent antigens. This isotype switching is known as **Class Switch Recombination** (CSR). Once this switch has occurred that particular B cell will usually no longer make the earlier isotypes, IgM or IgD.

T-independent activation of B-cells:

- Direct stimulation B-cells without T-helpers.
- Only IgM are produced.
- B memory cells are absent.

There are two types of T-cell-independent activation: type 1 of T-cell-independent (polyclonal) activation, and type 2 of T-cell-independent activation. Most important antibodies are produced (by T-cell-independent activation) against polysaccharide capsules of encapsulated bacteria.

Type 1 T-cell-independent activation occurs when a B-cell binds to an antigen and receives secondary activation by toll-like receptors. The resulting activated B-cell is restricted to IgM antibodies.

Type 2 T-cell-independent activation occurs when antigens that are expressed on the surface of pathogens with an organized and repetitive form can activate specific B-cells by the cross-linking of antigen receptors in a multivalent fashion. Many bacteria have repeating carbohydrate epitopes that stimulate B-cells, cross-linking their IgM antigen receptors, leading to IgM synthesis in the absence of T-cell stimulation.

Many antigens are T-cell-independent in that they can deliver both of the signals to the B-cell.

Cells of AMI:

1. **APCs:** macrophages, dendritic cells and B-cells.
2. **Effector cells**: plasma cells.
3. **Regulatory cells**: T-helpers cells (Th2 → IL-2, 4, 5, 6, 10, and 13).
4. **Memory B-cells** → secondary immune response.

Main stages of AMI (with T-dependent Ag):

1. Processing and presentation of Ag by APC bound with MHC class II to T helpers for recognization. B-cells, which possess BCR and MHC class II, can also present antigens to T-helpers, particularly during the secondary immune response.

2. Activated **Th2 cells** form IL-2 and other cytokines (IL–4, 5, 6, 10, 13) required for B-cells stimulation.

3. **B-cells** which have combined with their Ag proliferate (formation of a clone) and differentiate into the plasma cells and B memory cells.

4. **Plasma cells** begin to produce antibodies against antigen.

5. **B memory cells** are also forming; they remain fallowing an infection and are ready to develop immune response more rapidly.

Effector mechanisms of Ab (for elimination of Ag):

1. Immune phagocytosis (opsonization).
2. Complement-dependent lysis.
3. ADCC.
4. Neutralization of toxins and virus-infected cells.

Primary immune response is developing after first contact with antigen.

Characteristics:

- Slow in onset (latent period 3–5 days before Ab become detectable and within 15–30 days reach effective concentration for protection).
- Low concentrations of Ig are forming.
- "Short lived" (after short time — 1–6 months — Ab level declines).
- IgM are produced only.

Secondary immune response is developing after repeated contacts with same antigen.

Characteristics:

- Rapid in onset (several hours or 1–2 days).
- High concentrations of Ig are forming.
- "Long lived" (Ab remain detectable for months or years).
- IgG are produced mainly (in allergy — IgE, locally — sIgA).

Polyclonal and monoclonal antibodies

Antibodies that arise in an animal in response to a single complex antigen are heterogeneous because they are formed by several different clones of cells, each expressing an antibody capable of reacting with a different antigenic determinant on the complex antigen. These antibodies are said to be **polyclonal**.

Antibodies that arise from a single clone of cells, eg, in a plasma cell tumor (myeloma), are homogeneous and are referred to as monoclonal. Monoclonal antibodies can be produced by fusing a myeloma cell with an antibody-producing lymphocyte (plasma cell). Such hybridomas produce virtually unlimited quantities of monoclonal antibodies in vitro.

Application of monoclonal antibodies:

✓ Diagnostic tests (once monoclonal antibodies for a given substance have been produced, they can be used to detect the presence of this substance).

✓ Therapeutic treatment (therapeutic monoclonal antibodies act through a number of mechanisms, such as blocking of targeted molecule functions, inducing apoptosis of cells which express the target, or by modulating signalling pathways).

✓ Cancer treatment (one possible treatment for cancer involves monoclonal antibodies that bind only to cancer cell-specific antigens and induce an immunological response against the target cancer cell).

✓ Autoimmune diseases (monoclonal antibodies used for autoimmune diseases such as rheumatoid arthritis, ulcerative colitis by their ability to bind to and inhibit TNF-α; others inhibit IL–2 on activated T cells and thereby help pre-

vent acute rejection of kidney transplants; some Ab inhibit human IgE and is useful in allergic asthma therapy).

Lecture 8. ALLERGY. IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY OF THE INFECTIOUS DISEASES

ALLERGY (hypersensitivity to Ag) is a super-efficient immune response. Antigen which causes allergic reaction is called **allergen**.

Classification of allergens: **exoallergens** (infectious and noninfectious) and **endoallergens** (extra barrier tissues antigens, autoallergens).

After first contact with allergen **sensitization** is developed (immune cells are activated and immunoglobulins are sensitized), but only after second (repeated) contact with the same allergen **clinical symptoms** of allergy will be appeared and damage of the tissues and organs is present.

CLASSIFICATION OF ALLERGIC REACTIONS

• **Immediate type of hypersensitivity** (ITH) is called so because it is developing within 20–30 minutes after contact with the allergen and includes several subtypes:

1 type — **Actually immediate hypersensitivity (anaphylaxis).**

2 type — **Cytotoxic hypersensitivity.**

3 type — **Immune complex hypersensitivity.**

All of these subtypes are antibody-mediated (effector cells are B cells).

• **Delayed type of hypersensitivity** (DTH) that gets its name from the long time (48–96 h) that it takes for a skin reaction to Ag. It is cell-mediated type of allergy (effector cells are macrophages, T effector of DTH, T-helpers).

Actually immediate hypersensitivity

• First contact with allergen → activation of B-cells → production of IgE → Ig E is binding with basophiles and mast cells → mast cells are sensitized and lying in wait for second contact with allergen.

• Second contact with allergen → allergen interacts with IgE which connected with mast cells → degranulation of mast cells → release of mediators of inflammation (histamine, heparin, prostaglandin, etc) → physiological response (smooth muscles contraction, increase of vascular permeability, mucous secretion, etc).

Types of the clinical forms of anaphylactic hypersensitivity:

1. Localized anaphylaxis (atopic allergy).

• Allergens: pollen, fungal spores, dust mites, suspected food.

• Examples: Hay fever (allergic rhinitis), allergic conjunctivitis, bronchial asthma, true food allergy, urticaria (hives).

2. Systemic anaphylaxis (generalized response).

• Allergens: penicillin, antisera, venom of bees and wasps.

• Examples: Anaphylactic shock.

ATOPY is hereditary predisposition to hyper production of IgE to allergens.

Cytotoxic hypersensitivity

• First contact with allergen → activation of B-cells → production of IgM

and IgG → binding of IgM and IgG with different cells of body (mainly: blood cells) → cells are sensitized.

• Second contact with allergen → production of organ (tissue)-specific Ab.

• Mechanisms of destruction of the tissues:

➢ Complement-mediated cytotoxicity.

➢ ADCC (by K-cells).

• Examples of the clinical forms: drug allergy, hemolytic disease of newborn, autoimmune hemolytic anemia, leucopenia, thrombocytopenia, autoimmune diseases of the different organs.

Immune complex hypersensitivity

• Involves Ab (IgM and IgG) that react with free antigens → **circulating immune complexes** (CIC) → deposition of CIC in organs and tissues (most common kidney, skin, blood vessels, and joints) → activation of complement → tissue damage and inflammation.

• Examples of the clinical forms: Arthus reaction, serum sickness, pneumonitis, rheumatoid arthritis, glomerulonephritis, SLE (systemic lupus erythematosus).

Delayed hypersensitivity (cell-mediated hypersensitivity)

• T-cell-mediated immune response to allergens.

• Allergens of DTH:

1. **Infectious**: intracellular bacterial pathogens (Mycobacteria); viruses, fungi, protozoa ► chronic infections with DTH in pathogenesis (infectious allergy).

2. **Noninfectious**: poison ivy, nickel, formaldehyde, latex, dyes in clothing and cosmetics (they are haptens).

Examples of the clinical forms: skin tests (Tuberculin test), certain chronic bacterial infections (TB, leprosy), contact dermatitis.

Laboratory diagnosis of allergic reactions

• 1 type: skin tests (registration within 20 min) — revealing of IgE.

• 2 type — Revealing of Ag to blood cells.

• 3 type — Revealing of CIC.

• 4 type — Skin allergic tests.

The archetype of delayed hypersensitivity is the tuberculin reaction. When a small dose of tuberculin is injected intradermally in an individual sensitized to tuberculoproteins by prior infection or immunization, an indurated inflammatory reaction develops at the site within 48–72 hours. In unsensitized individuals, the tuberculin injection provokes no response. The tuberculin test therefore provides useful indication of the state of DTH to the bacilli. The tuberculin test differs from the skin test for Type I hypersensitivity not only in the longer interval for appearance but also in its morphology and histology.

Tuberculin type hypersensitivity develops in many infections with bacteria, fungi, viruses and parasites, especially when the infection is subacute or chronic

and the pathogen intracellular. A similar hypersensitivity is developed in allograft reaction and in many autoimmune diseases.

IMMUNOPROPHYLAXIS (IMMUNIZATION)

Prevention of the infectious diseases by the creation of different types of the artificial immunity in the human body.

Forms of immunoprophylaxis:

Routine immunization (schedules have been developed for different countries → "Vaccine preventable diseases": diphtheria, pertussis, tetanus, polio, measles, mumps, rubella, tuberculosis).

Bacterial vaccines in childhood immunization schedule:

✓ DPT ► diphtheria, pertussis (whooping cough), tetanus vaccine;
✓ Hib ► Haemophilus influenzae type b vaccine;
✓ PCV ► pneumococcal vaccine;
✓ BCG ► Tuberculosis (TB) vaccine;
✓ OPV ► oral polio vaccine (Sabin vaccine);
✓ IPV ► inactivated polio vaccine (Salk vaccine);
✓ MMR ► measles, mumps and rubella vaccine;
✓ HBV ► hepatitis B vaccine;
✓ Varicella ► varicella (chicken pox) vaccine.

Individual immunization (typhoid vaccine, varicella vaccine, hepatitis B vaccine, cholera vaccine) can be used in the cases:

✓ in endemic regions;
✓ after suspected bites;
✓ for laboratory and medical workers;
✓ individual predisposition.

Types of immunoprophylaxis:

1. Active immunoprophylaxis (used vaccines → active artificial immunity).
2. Passive immunoprophylaxis (used prophylactic antisera → passive artificial immunity).

VACCINES

Vaccine is a preparation which contains the microbes (bacteria, viruses) or their components (pure Ag, toxin, genes, ribosomes) and capable to produce the active artificial immunity. Application of vaccines: immune prophylaxis (vaccination) and treatment (vaccinotherapy).

Effective vaccine:

• Must be safe.
• Provides long-term protection.
• Must be immunogenic (can induce high level of Ab formation).
• Must be biologically stable (areactive).
• Cheap to produce.
• Easy to administration.

Types of the vaccines:

• Monovaccines (includes one species).

• Combined vaccines (DPT, MMR).

• Monovalent, trivalent, heptavalent (number of serotypes).

• Conjugate vaccined (polysaccharide microbial Ag + «conjugate» such as tetanus toxoid).

Classification of the vaccines:

1. Live attenuated vaccine (bacterial and viral).
2. Inactivated (killed) vaccine.
3. New generation of killed vaccines are fractional vaccines (consist of purified microbial subunits):
   - ✓ Subvirion vaccine.
   - ✓ Surface Ag (protective Ag).
   - ✓ Toxoid (anatoxin).
   - ✓ Capsular polysaccharides.
   - ✓ Recombinant vaccines (genetically-engineered vaccines).
4. DNA-vaccines (viral gene + plasmid + promoter).
5. Anti-idiotype-specific monoclonal Ab.

Characteristics of the live vaccines:

- It is attenuated (weakened) form of the "wild" virus or a bacterium.
- Immune response similar to the natural infection → long-lived immunity.
- Usually produce immunity with one dose, except those administered orally.
- Severe reactions possible (allergy, disease, convulsions, high fever).
- Unsafe in immunocompromised persons and during pregnancy.
- Fragile (must be stored and handled carefully).

Characteristics of the killed vaccines:

- Produced by inactivating of the microbes.
- In the case of fractional vaccines, microbe is further treated to purify only those components to be included in the vaccine (e.g., the polysaccharide capsule of pneumococcus).
- Less immunogenic but more safe than live vaccines.
- Generally require 3–5 doses (booster doses).
- Immune response mostly humoral (Ab titer diminishes with time).
- Need addition of “adjuvants” ($AlPO_3$) for toxoids.
- May be administered parenterally.
- Killed vaccines contain the microbes which inactivated by heating, UV, chemicals but not denaturated.
- Toxoid (anatoxin) is inactivated exotoxin by heating; it is not virulent but stays immunogenic.

Booster shots

Repeated inoculations of vaccines to maintain a high titer (level) of Ab.

Example: *epidemics in school may prompt recommendations for specific boosters.*

Creation of recombinant vaccines

• Cloning of genes providing synthesis of necessary antigens.

• Introduction of these antigens into vector.
• Introduction of vectors into the recipient cells.
• Culture of recombinant cells *in vitro*.
• Isolation of antigen and its purification.

Complications of vaccination:

✓ Autoimmune reactions.
✓ Development of disease in immunosuppressed individuals.
✓ Contra-indications for vaccination (cancers, pregnancy).
✓ Hypersensitivity.

Vaccine development has several trends:

• Combinations of vaccines are becoming more common; vaccines containing five or more components are used in many parts of the world. In 2013, *Biofarma* has released a new product called *Pentabio*, which is combination vaccine of diphtheria, tetanus, pertussis, hepatitis B, and *Haemophilus influenzae* for baby/infant.

• New methods of administering vaccines are being developed, such as skin patches, aerosols via inhalation devices, and eating genetically engineered plants.

• Vaccines are being designed to stimulate innate immune responses, as well as adaptive.

• Attempts are being made to develop vaccines to help cure chronic infections, as opposed to preventing disease.

• Vaccines are being developed to defend against bioterrorist attacks such as anthrax, plague, and smallpox.

PASSIVE IMMUNOPROPHYLAXIS

Transfer of preformed Ab from the donor (animal or human) to the recipient. Passive immunity can occur *naturally*, when maternal antibodies are transferred to the fetus through the placenta (IgG) or with breast milk (sIgA), and can also be induced *artificially*, when high levels of human (or horse) antibodies specific for a pathogen or toxin are transferred to non-immune individuals. Passive immunization is used when there is a high risk of infection and insufficient time for the body to develop its own immune response, or to reduce the symptoms of ongoing or immunosuppressive diseases. Artificially acquired passive immunity is a short-term immunization (immunity lasts for a few weeks or months). Passive immunity provides immediate protection, but the body does not develop memory, therefore the patient is at risk of being infected by the same pathogen later. There is also a potential risk for hypersensitivity reactions, and serum sickness, especially from gamma globulin of non-human origin.

Preparations for passive immunization:

1. Human or animal blood plasma or antisera.

2. Pooled human immunoglobulin for intravenous (IVIG) or intramuscular (IG) use. It contains the pooled, polyvalent, IgG antibodies extracted from the plasma of over one thousand blood donors. IVIG's effects last between 2 weeks and 3 months.

3. High-titer human IVIG or IG from immunized donors or from donors re-

covering from the disease (hyperimmune Ig against specific Ag).

4. Monoclonal antibodies.

Classification of sera

1. Heterologous antisera (hyper immunization of animals/horses by Ag).

2. Homologous antisera (from human donors convalescing from natural infections or donors artificially immunized).

Types of antisera

- ✓ Antibacterial.
- ✓ Antitoxic.
- ✓ Antiviral.
- ✓ Anti Rh^+ or Ig (D).
- ✓ Anti-venom (snake, bees) or Antivenins.

Immunoglobulins are Ab-containing solution derived from human blood serum obtained by cold ethanol fractionation of large pools of plasma. They may be intravenous and intramuscular preparations. Methods of receiving: from human donors that had been suffered from certain infections or after active immunization; also from post-abortion tissues.

Examples of antisera and Ig wide used in practical medicine:

- ✓ Diphtheria antitoxin.
- ✓ Tetanus Ig.
- ✓ Hepatitis B Ig.
- ✓ Rabies Ig.

Application of antisera and Ig:

(1) Postexposure and preexposure prophylaxis (passive immunization).

(2) Immunotherapy:

- ✓ Use for immunotherapy of the chronic infections or in emergency for prevention and treatment of certain diseases (tetanus, tick-born encephalitis, diphtheria, botulism).
- ✓ Treatment of immunodeficiencies and autoimmune diseases.

Complications of passive immunization (immunotherapy):

- Hypersensitivity (anaphylactic shock, serum sickness).
- Rick of HIV transmission.
- Rick of prion transmission.

IMMUNOTHERAPY

Influence on immune system with the purpose of stopping of the pathological process.

Preparations for immunotherapy:

1. Vaccines (vaccinotherapy involves activating the immune system to respond to an infectious agent).

2.Therapeutical and prophylactic antisera or immunoglobulins.

3. Immunomodulators (active agents of immunotherapy are collectively called immunomodulators such as IL–2, IL–7, γ–IFN, CSF, chemokines CCL3,

CCL26, CXCL7, etc.).

Immunomodulators

➢ *Activating immunotherapy* (cancer treatment).

Cell-based immunotherapy is based on use of immune effector cells such as lymphocytes, macrophages, dendritic cells, NK cells, CTL, etc.

- Dendritic cell-based immunotherapy (dendritic cells can be stimulated to activate CMI towards an antigen *in vitro*; upon transfusion back into the patient these activated cells present tumor antigen to effector lymphocytes and provoke specific CMI).
- Adoptive cell transfer uses T-cell-based cytotoxic responses to attack cancer cells. T cells that have a natural or genetically engineered reactivity to a patient's cancer are generated *in vitro* and then transferred back into the cancer patient.
- Autologous immune enhancement therapy (AIET) is wherein the patient's own peripheral blood-derived NK cells, CTLs and other relevant immune cells are expanded *in vitro* and then reinfused to tackle cancer.
- Genetically engineered T-cells are created by infecting patient's cells with a virus that contain a copy of a T-cell receptor (TCR) gene that is specialized to recognize tumor antigens. The virus is not able to reproduce within the cell however integrates into the human genome. This is beneficial as new TCR gene remains stable in the T-cell. A patient's own T cells are exposed to these viruses and then expanded non-specifically or stimulated using the genetically engineered TCR. The cells are then transferred back into the patient and ready to have an immune response against the tumor.

Immune suppression dampens an abnormal immune response in autoimmune diseases or reduces a normal immune response to prevent rejection of transplanted organs or cells.

➢ *Immunosuppressive immunotherapy*.

- Immunosuppressive drugs are important tools in the management of organ transplantation and autoimmune disease. Immune responses depend on lymphocyte proliferation, and cytostatic drugs are immunosuppressive. *Glucocorticoids* are somewhat more specific inhibitors of lymphocyte activation.
- Immune tolerance is the process by which the body naturally does not launch an immune system attack on its own tissues. An immune tolerance therapy seeks to reset the immune system so that the body stops mistakenly attacking its own organs or cells in autoimmune disease or accepts foreign tissue in organ transplantation.
- Allergy treatment. While other allergy treatments (such as antihistamines or corticosteroids) treat only the symptoms of allergic disease, immunotherapy is the only available treatment that can modify the natural course of the allergic disease, by reducing sensitivity to allergens. The therapy is indicated for people who are extremely allergic or who cannot avoid specific allergens. For example, they may not be able to live a normal life and completely avoid pollen, dust mites, mold spores, pet dander, insect venom, and certain other common triggers of allergic reactions. Immunotherapy is generally not indicated for food or me-

dicinal allergies. This therapy is particularly useful for people with allergic rhinitis or asthma. Immunotherapy involves a series of injections (shots) given regularly for several years by a specialist in a hospital clinic (allergy extract).

Lecture 9. CLINICAL IMMUNOLOGY. TOLERANCE. TRANSPLACENTAL AND ANTITUMORAL IMMUNITY. AUTOIMMUNE DISEASES. IMMUNODEFFICIENCIES

TOLERANCE

Tolerance is absence of the specific immune response in a presence of available specific Ag (*unresponsiveness*). Ag is called **tolerogen.**

Types of tolerance:

- Natural (tolerance to own self Ag) → acquired during the fetal life.
- Induced → acquired during a life.

Development of tolerance:

- Contact of fetus with Ag during the prenatal life.
- High doses of circulating Ag.
- Molecular mimicry: resemblance between bacterial Ag and host Ag.

Mechanisms of tolerance:

1. Deletion of clones of B and T-cells by apoptosis (negative selection).
2. Inactivation of BCR and TCR.
3. Stopping the proliferation of the lymphocytes.

Application of tolerance in medicine:

- Depression of immunity during transplantation.
- Depression of autoimmune reactions.
- Treatment of allergy.

TRANSPLANTATION IMMUNITY

Transplantation (grafting) is process of moving cells, tissues, organs from donor to recipient. Object that is transplanted is called **graft.**

Types of graft:

• Autograft — from one individual to same.

• Syngeneic graft — from one identical twin to other.

• Allograft — from one genetically different individual to other of the same species.

• Xenograft — between two individuals of different species.

Graft rejection (GR) is a reaction of the recipient immune system against graft of donor (MHC antigens control the reaction of graft rejection).

Mechanism of GR:

✓ CMI (CTL with CD8 recognize MHC I class of the transplant cells and cause specific cytotoxicity of graft).

✓ AMI is additional mechanism.

Types of GR:

• Hyper acute (within minutes or hours).
• Acute (after 1 week).
• Chronic (after several months).

Methods of stopping the GR:

1. Immunosuppression.
2. Detection of pre-existing Ab to graft.
3. Tissue typing (for HLA).
4. Induction of tolerance of recipient to graft.

GvHD (Graf-versus-Host Disease) or **GVH reaction** is immune response of new graft against bone marrow of host.

ANTITUMORAL IMMUNITY

Tumor is uncontrolled growth of tissue that affects homeostasis of organism and eventually kills this organism.

Malignant tumors are called **cancers.**

Tumor antigens:

- Tumor-specific Ag (TSA) — unique for tumor only.
- Tumor-associated Ag (TAA) — abnormal cell Ag.

Mechanisms of oncogenesis:

1. Mutation of host genes.
2. Activation of tumor suppressor genes of host.
3. Oncogenic viruses.

Immunity to tumors is provided by CTLs**,** ADCC, NK-cells and macrophages. Tumors cam inhibit immune response of host.

IMMUNOPATHOLOGY

Immunopathology includes:

1. Hypersensitivity (see previous lecture);
2. Autoimmune diseases (autoimmunity);
3. Immunodeficiency.

AUTOIMMUNITY AND AUTOIMMUNE DISEASES

Autoimmunity is immune response against self Ag (“self” is recognized by the immune system as “non-self”).

- Basis — breakdown of natural tolerance.
- Ag is called **autoantigen.**
- Mechanisms — AMI and CMI.

Autoimmune reaction develops during:

1. Damage of autoantigens.
2. Immune response on cross-reactive antigens.
3. Releasing of Ag during injury that in norm are isolated from immune system (extra barrier tissue Ag — localized in brain, testicles, anterior chamber

of eye, crystalline lens).

4. Misbalance of regulation of immune system (atypical presentation, insufficient suppression, etc).

Classification of autoimmune diseases:

1. *Systemic autoimmune diseases*:
   - ✓ Rheumatoid arthritis.
   - ✓ Systemic Lupus Erythematosus (SLE).
   - ✓ Goodpasture syndrome.
2. *Organ-specific autoimmune diseases*:
   - ✓ Multiple sclerosis.
   - ✓ Myasthenia gravis.
   - ✓ Diabetes.
   - ✓ Hepatitis.
   - ✓ Thrombocytopenia.
   - ✓ Hemolytic anemia.

IMMUNODEFICIENCIES ("defective immunity")

Disorder of immune system when it cannot to protect of the organism against pathogens and tumor cells.

Classification of immunodeficiency:

1. Primary (congenital) immunodeficiencies (genetic, hereditary disorders of immune system).
2. Secondary (acquired) immunodeficiencies (more common than primary; acquired during life due different factors).
3. Cellular, humoral or combined immunodeficiencies.

Congenital (primary) immunodeficiency

1. Congenital immunodeficiency of <u>specific immunity</u> (CMI, AMI):
   - ✓ **Immunodeficiency of B-cells** (X-linked or Bruton's agammaglobulinemia → absence of Ig and B-cells, hypogammaglobulinemia, IgG deficiency).
   - ✓ **Immunodeficiency of T-cells** (DiGeorge syndrome → absence of thymus and T-cells).
   - ✓ **Severe Combined Immunodeficiency** (SCID).

2.Congenital immunodeficiency of <u>innate immunity</u>:
   - ✓ Defects of phagocytes.
   - ✓ Defects of NK cells.
   - ✓ Defects of complement.

Example: chronic granulomatous disease, complement deficiency.

Factors leading to secondary immunodeficiency:

- ✓ Infections (influenza, HIV, rubella, hepatitis, TB, syphilis).
- ✓ Chronic noninfectious diseases.
- ✓ Malnutrition.

- ✓ Ionizing radiation.
- ✓ Tumors.
- ✓ Immunosuppressive drugs and hormones.
- ✓ Unhealthy mode of life.
- ✓ Distress, depression, trauma.
- ✓ Burns, long bleedings.

Examples of secondary immunodeficiencies

- Common hypogammaglobulinemia.
- AIDS (acquired immune deficiency syndrome).
- Neutropenia.
- Chronic Fatigue Immune Dysfunction Syndrome.
- Chronic pyoseptic infections of skin, RT, GIT, urogenital system.

❖ **X-linked agammaglobulinemia** (XLA) is a rare X-linked genetic disorder/mutation (more common in males). XLA patients do not generate mature B-cells, which manifests as a complete lack of antibodies in their bloodstream. Patients with untreated XLA are prone to develop serious and even fatal infections. Patients typically present in early childhood with recurrent infections, in particular with extracellular, encapsulated bacteria. XLA is treated by infusion of human antibody. Treatment with pooled gamma globulin cannot restore a functional population of B cells, but it is sufficient to reduce the severity and number of infections due to the passive immunity granted by the exogenous antibodies. XLA was first characterized by Dr. Ogden Bruton. It is the first known immune deficiency, and is classified with other inherited (genetic) defects of the immune system, known as primary immunodeficiency disorders.

❖ **Hypogammaglobulinemia** (or agammaglobulinemia) implies that gamma globulins are reduced or completely absent. Hypogammaglobulinemia should be distinguished from *dysgammaglobulinemia*, which is a reduction in some types of gamma globulins, but not others.

❖ **DiGeorge syndrome** (DGS) is a syndrome caused by the deletion of a small piece of chromosome 22. The inheritance pattern is autosomal dominant and it has a prevalence estimated at 1:4000. The syndrome was described in 1968 by the pediatric endocrinologist Angelo DiGeorge. The features of this syndrome vary widely, even among members of the same family, and affect many parts of the body. Characteristic signs and symptoms may include birth defects such as congenital heart disease, defects in the palate, most commonly related to neuromuscular problems with closure, learning disabilities, mild differences in facial features, and recurrent infections. Affected individuals may also have any other kind of birth defect including kidney abnormalities and significant feeding difficulties as babies. Disorders such as hypothyroidism and hypoparathyroidism or

thrombocytopenia, and psychiatric illnesses are common late-occurring features.

❖ **Chronic granulomatous disease** (CGD) is a diverse group of hereditary diseases in which certain cells of the immune system have difficulty forming the reactive oxygen compounds (most importantly, the superoxide radical) used to kill certain ingested pathogens. This leads to the formation of granulomata in many organs. Classically, patients with chronic granulomatous disease will suffer from recurrent bouts of infection due to the decreased capacity of their immune system to fight off disease-causing organisms. The recurrent infections they acquire are specific and are, in decreasing order of frequency: pneumonia, abscesses of the skin, tissues, and organs, suppurative arthritis, osteomyelitis, bacteremia/fungemia, and superficial skin infections such as cellulitis or impetigo. Early diagnosis is important since these people can be placed on antibiotics to ward off infections before they occur.

❖ **Complement deficiency** is an immunodeficiency of absent or suboptimal functioning of one of the complement system proteins. The disorders can be divided into two categories: (1) disorders of the proteins that act to inhibit the complement system (such as C1-inhibitor) can lead to an overactive response, causing conditions such as hereditary angioedema and hemolytic-uremic syndrome (HUS); (2) disorders of the proteins that act to activate the complement system (such as C3) can lead to an underactive response, causing greater susceptibility to infections. The total hemolytic complement *CH50 level* in the blood will be low or undetectable with complement deficiencies. A vaccination for encapsulated organisms is crucial for preventing infections in complement deficiencies.

Lecture 10. GRAM-POSITIVE AND GRAM-NEGATIVE COCCI

STAPHYLOCOCCI

TAXONOMY

Domain: Bacteria
Phylum: Firmicutes
Class: Bacilli
Oder: Bacillales
Family:Staphylococcaceae
Genus:**Staphylococcus**

The three main species of clinical importance are *Staphylococcus aureus, S.epidermidis* and *S.saprophyticus.*

MORPHOLOGY

- **Gram-positive cocci** in clusters(as a "vine grapes"); nonmotile, nonsporing, forming *in vivo* polysaccharide microcapsule. They may also be found singly, in pairs, tetrads and in short chains, especially when examined from liquid culture. Long chains are never present.
- **Protein A** is in a cell wall.

BIOLOGICAL PROPERTIES

- Facultative anaerobes
- Mesophiles
- **Salt tolerant** (halotolerant)

MEDIA FOR CULTIVATION AND CULTURAL PROPERTIES

• Staphylococci can grow on simple media MPB and MPA.
• Blood agar → hemolytic colonies (β-hemolysis is present).
• Yolk-salt agar (YSA) and mannitol-salt agar are selective media.
• *S.aureus* usually forms golden (yellow) S-colonies (carotenoid pigments).
• *S.aureus* possesses lecithinase and, when grown on YSA, produces*lecithinase-positive colonies with aura*.
• In liquid media (salt MPB), diffusive turbidity is produced.

BIOCHEMICAL ACTIVITY

- Staphylococci ferment a number of sugars, producing acid but no gas. Sugar fermentation is of no diagnostic value except of mannitol, which is usually fermented anaerobically by *S.aureus* but not by other species.
- They are catalase positive and oxidase negative (unlike streptococci).

ANTIGENIC STRUCTURE

- Cell wall components (peptidoglycan, teichoic acids, protein A) and exotoxins (especially α-toxin). Protein A is a marker for *S.aureus* and can be detected in reaction of precipitation or latex-agglutination.
- Serotyping is not used for routine microbiological diagnostics (typing of species is carried out with help of bacteriophages).

ECOLOGY AND RESISTANCE

- Facultative pathogens and extracellular parasites.
- Normal flora of skin, nose, oral cavity, mucous membranes.
- Staphylococci are relatively resistant bacteria to the factors of environment and they are distributive in nature.
- Hospital strains of *S.aureus* are often resistant to many antibiotics. **Methicillin-resistant *S.aureus***(MRSA) causes outbreaks in hospitals and can be epidemic. **Vancomycin-resistant *S.aureus*** (VRSA) refers to strains of *S.aureus* that have become resistant to the glycopeptide antibiotic *vancomycin*. *S.epidermidis* nosocomial isolates are also often resistant to several antibiotics.

FACTORS OF PATHOGENICITY

1. Factors of adhesion (teichoic and lipoteichoic acids).
2. Protein A (antiphagocytic and anticomplement activity).
3. Capsular polysaccharide (antiphagocytic).
4. Exotoxins:

- Membrane toxins − hemolysins (alpha, beta, gamma, delta toxins) and leucocidins. Majority of MRSA strains produce aexotoxin called Panton Valentine Leucocidin (PVL), which is associated with necrotizing infections.
- Enterotoxins (superantigens): responsible for food intoxication (8 serotypes: A-E, H, G and I). The exotoxinsare believed to act directly on CNS (activation of the vomiting center in brain), rather than on the gastrointestinal mucosa.
- **T**oxic **s**hock **s**yndrome **t**oxin (TSST); also a superantigen and is weakly related to enterotoxins but it does not have emetic activity (responsible for toxic shock syndrome).
- Exfoliative (epidermolytic) toxin or SSSST (**t**oxin is responsible for the "**s**taphylococcal **s**kin **s**calded **s**yndrome").

5. Enzymes of invasion:

- Plasmacoagulase/coagulase (*S.aureus* is in a group of **c**oagulase **p**ositive **s**taphylococci [CPS], which is more virulent and invasive; other staphylococcal species are considered as **c**oagulase **n**egative **s**taphylococci [CNS]); coagulase provides clotting of plasma, converting fibrinogen to fibrin. Fibrin is accumulated on the surface of staphylococci and provides antiphagocytic effect.
- Other enzymes: lecithinase, hyaluronidase, staphylokinase (fibrinolysin), β-lactamase.

CAUSED DISEASES

Staphylococcal diseases (*S.aureus*) can be divided into two groups:

❖ **Toxin-mediated diseases**(intoxications):

- Food intoxication is caused by enterotoxins, which are preformed in food, and hence has a short incubatory period (2-6 hours). Vomiting typically is more prominent than diarrhea. Meat and fish or milk and milk products (ice-cream) cooked and left at room temperature after contamination by staphylococci are the common factors of toxicosis.

• Toxic shock syndrome is potentially fatal multisystem disease presenting with fever, hypotension, myalgia, vomiting, diarrhea, mucosal hyperemia and erythematous rash.

• Staphylococcal skin scalded syndrome is exfoliative skin disease in which outer layer of epidermis gets separated from underlying tissue). The severe form of SSSS is known as Ritter's disease in the newborn and toxic epidermal necrosis in older patients. Milder forms are pemphigus neonatorum and bullous impetigo.

❖**Pyoseptic infections (PSI):**

• Skin and soft tissue → furuncle (boil), abscess, carbuncle, surgical-wound infections; eyelid infection (blepharitis), postpartum breast infection (mastitis).

• RT → tonsillitis, pharyngitis, sinusitis, otitis, pneumonia.

• CNS → meningitis.

• CVS → endocarditis(infection of the heart valves), sepsis.

• MSS → osteomyelitis, arthritis.

S.epidermidis colonizes catheters and heart valves → endocarditis (in drug addicts). *S.saprophyticus* causes cystitis in sexually active women.

EPIDEMIOLOGY

•Source of infection is sick persons or carriers. *S.aureus* is carried, usually transiently, in the anterior nares and on the skin.

•Mechanisms of transmission are airborne, contact, alimentary, inoculation (during medical operations) and autoinfection.

S.aureus is a frequent causative agent of many **nosocomial infections**.

IMMUNITY, TREATMENT AND PREVENTION

• Nonlong-lasting immunity.

• Antibiotic therapy: β-lactams (penicillins and cephalosporins). For resistant strains can be used beta-lactamase-resistant penicillins (e.g., oxacillin), specific inhibitors of β-lactamases (e.g., clavulanic acid or sulbactam) and other inhibitors of cell wall synthesis — carbapenems, glycopeptides, vancomycin or teicoplanin. As a drug resistance is so common among staphylococci, the appropriate antibiotic should be chosen based on antibiotic sensitivity tests.

• Aseptic actions (washing hands, sterilizing shared equipment); nasal and topical body decolonization is recommended.

• Chronic staphylococcal infections require specific immune therapy: staphylococcal autovaccine, antitoxic serum and anti-staphylococcal ɣ-globulin. For activation of anti-staphylococcal immunity the toxoid, derived from alpha-toxin, is administered.

STREPTOCOCCI

TAXONOMY

Domain: Bacteria
Phylum: Firmicutes
Class: Bacilli
Oder: Lactobacillales
Family: Streptococcaceae

Genus **Streptococcus** includes many species: *S.pyogenes, S.agalactiae, S.pneumoniae, S.mitis, S.mutans*, etc.

Genus **Enterococcus** includes several species: *E.faecalis, E.faecium*.

MORPHOLOGY

- **Gram-positive cocci** in chain;they are nonmotile and nonsporing, forming in vivo microcapsule (hyaluronic acid capsule for *S.pyogenes*, polysaccharide capsules for the most of other streptococci).
- Enterococci are typically appearing as pairs of oval cocci which arranged at an angle, or in short chains.
- Protein M is in cell wall.

BIOLOGICAL PROPERTIRES

- Facultative anaerobes.
- Mesophiles.

MEDIA FOR CULTIVATION AND CULTURAL PROPERTIES

- Streptococci can't grow on simple media.
- Blood agar; serum agar, sugar MPB and MPA are common media.
- S-colonies with different types of hemolysis: alpha hemolysis → partial (greenish zone of hemolysis, these streptococci are known as "**viridans streptococci**" — from "*viridis*" meaning green); beta hemolysis → complete (clear zone of hemolysis, these streptococci are called "**beta-hemolytic streptococci**"), gamma hemolysis → "**nonhemolytic**" streptococci.
- In liquid media growth → granular turbidity with a powdery deposit.

BIOCHEMICAL ACTIVITY

- Streptococci ferment several sugars producing acid but no gas.
- Streptococci are catalase and oxidase negative.
- PYR test (hydrolysis of pyrrolidonyl naphthylamide) is useful for differentiating of *S.pyogenes* from other streptococci.
- Enterococci have ability to grow in the presence of bile, 6,5 % of NaCl, hydrolyze esculin that distinguishes them from other streptococci.

ANTIGENIC STRUCTURE

Streptococcal antigens include hyaluronic acid capsule, cell wall components and exotoxins. Classification of streptococci is based on antigenic properties and type of hemolysis.

CLASSIFICATION OF STREPTOCOCCI

Streptococci are classified by Rebecca Lancefield (1933) serologically into 20 groups (Lancefield groups) on the nature of a carbohydrate antigen (C substance or Lancefield antigen) occurring in their cell walls. Most important pathogens are present in A, B and C groups. Certain groups can be divided into serotypes on the base of M, R and T protein antigens present on cell surface (Griffith typing). The great majority of hemolytic streptococci that produce human infections belong to group A (*S.pyogenes*). *S.pyogenes* group consists of 80 serotypes.

Table 13 — Medically important streptococci and their characteristics

Group	Species	Predominant type of hemolysis	Localization of normal flora
Group A streptococci (GAS)	*S.pyogenes**	Beta-hemolysis	Upper respiratory tract, skin
Group B streptococci (GBS)	*S.agalactiae*	Beta-hemolysis	Female genital tract (GBS increase the risk for premature rupture of membranes during pregnancy)
Group C	*S. equisimilis*	Beta-hemolysis	Throat
Group D *Nonenterococcal group D includes *S.bovis*	Enterococci: *E. faecalis* *E. faecium*	Gamma hemolysis (nonhemolytic)	Colon
Non-typed streptococci:			
Viridansstreptococci: *S.salivarius, S.sanguis* *S.mutans, S.mitis*		Alpha-hemolysis (greenish)	Mouth ("oral streptococci"), colon, female genital tract
Pneumococci: *S.pneumoniae*		Alpha-hemolysis	Upper respiratory tract

* *S.pyogenes* is denoted as **g**roup **A**β-**h**emolytic **s**treptococci (**GABHS**).

Various structural components of *S.pyogenes* (**cross-reactive antigens**) provide antigenic cross reactions with different tissues of human body. Antigenic relationships ("antigenic mimicry") are demonstrated between capsular hyaluronic acid and synovial fluid; cell wall M protein and myocardium; carbohydrate C and cardiac valves. It is believed that these antigenic cross reactions may account for some cases of *acute rheumatic fever* and other streptococcal diseases of autoimmune origin.

ECOLOGY AND RESISTNCE

- Facultative pathogens and extracellular parasites.
- Normal flora of skin, nose, oral cavity, female genital tract, intestinal tract, mucous membranes.
- Streptococci are relatively resistant in nature. But they sensitive to many antibiotics unlike *S.aureus*. Sensitivity to bacitracin is employed as a convenient method for differentiating *S.pyogenes* from other hemolytic streptococci.

FACTORS OF PATHOGENICITY

1. Protein M (antiphagocytic and anticomplement activity).
2. Exotoxins:

• Hemolysins (streptolysins O and S). Streptolysin O is so called because it is oxygen labile. It destroys the membranes of RBCs and others. Estimation of **a**nti**s**treptolysin **O**Ab titer (ASO titer) is a standard serological test for the retrospective diagnosis of the streptococcal infections. Streptolysin S is oxygen stable and not antigenic.

• **S**treptococcal **p**yrogenic **e**xotoxin – SPE (Erythrogenic, Dick toxin, scarlatinal toxin). Effects of the toxin are is erythemous reaction (rash) and fever.

SPE is associated with streptococcal toxic shock syndrome and scarlet fever. It is superantigen and encoded by specific phage.

3. Enzymes of invasion:

• Streptokinase (fibrinolysin) promotes the breaking down the fibrin barrier around the streptococcal lesions and facilitates the spread of infection. Streptokinase is given intravenously for the treatment of pulmonary emboli of coronary artery and venous thrombosis. Anti-streptokinase Ab also provide retrospective diagnosis of the streptococcal infections.

• Streptodornase (DNAase) helps to liquefy the pus (large amount of DNA derived from the nuclei of necrotic cells).

• Other enzymes: hyaluronidase, proteinase, phosphatase, neuraminidase.

CAUSED DISEASES

Streptococcal infections are diffuse and rapidly spreading those involve the tissues and extend along lymphatic pathways with only minimal local suppuration (unlike staphylococci).

Table 14 — Role of streptococci in human pathology

Species	Type of pathogenesis	Typical diseases
S.pyogenes (**group A**)	**PSI**	Impetigo (pyoderma), erysipelas (massive edema), cellulitis, necrotizing fasciitis,* pharyngitis (strep throat), tonsillitis (quinsy or angina), peritonsillar abscess, otitis, conjunctivitis, endocarditis, sepsis, puerperal fever (sepsis after childbirth)
	Toxin-mediated	Scarlet fever,** toxic shock syndrome
	Immunogenic	Acute rheumatic fever (disease that affects the joints and heart valves), acute glomerulonephritis (affection of kidney)***
S.agalactiae (**group B**)	PSI	Neonatal sepsis and meningitis
Enterococci **group D** (*E. faecalis, E.faecium*)	PSI	Urinary tract infections, endocarditis, prostatitis, intra-abdominal infections, pelvic infections, wound infections, bacteremia
Viridans streptococci	PSI	Endocarditis, dental caries
Pneumococci	PSI	Pneumonia, otitis media, meningitis

* Necrotizing fasciitis (streptococcal gangrene) is extensive necrosis of subcutaneous and muscular tissue and fascia with toxic shock (more commonly caused by specific M types which produce SPE and these strains are known as "**flesh-eating streptococci**".

** Scarlet fever (older — scarlatina) is common children respiratory infection with pharyngitis, rapidly spreading trunk rash, fever, bright "strawberry" tongue, skin desquamation.

*** These diseases are believed to be the result of hypersensitivity III type to some streptococcal components. It has also been suggested that there may be an element of autoimmunity is involved, and antigenic cross-reactions have been demonstrated between streptococcal antigens and host tissues (antigenic mimicry).

EPIDEMIOLOGY

- Source of infection is sick persons or carriers (especially important when carriers are in areas such as obstetric delivery rooms, operating rooms).
- Mechanisms of transmission are airborne, contact, autoinfections.

IMMUNITY, TREATMENT AND PREVENTION

• Nonlong-lasting immunity (but after scarlet fever is long-lasting).

• Antibiotic therapy is required due to prevention of post-streptococcal complications such as rheumatic fever. Commonly in use beta-lactams, macrolides, fluoroquinolones. Penicillin is a drug of choice.

• Enterococci are resistant to multiple drugs, ex.: penicillins, aminoglycosides and vancomycin.

• **Vancomycin resistant enterococci** (VRE) now are important cause of nosocomial infections.

• No vaccination.

Pneumococci

- *Streptococcus pneumoniae* is habitat of URT.
- **Gram-positive** lancet-shaped **diplococci** with polysaccharide capsule.
- Over 90 serotypes of *S.pneumoniae*are differentiated by K-Ag.
- Aerobes and capnophilic; alpha-hemolysis.
- Tests for detection: inhibition by optochin and lysis by bile.
- Factors of pathogenicity: protein M, capsule, IgA-protease, pneumolysin (streptolysin O).
- Caused diseases (PSI): **bronchopneumonia** or **lobar pneumonia,** acute purulent **meningitis,** acute **otitis media, paranasal sinusitis, sepsis/bacteremia, endocarditis, septic arthritis**, etc.
- Source of infection is sick persons and carriers. Pneumococci transiently colonize the pharynx and microbocarriage in healthy people has been reported to be as high as 75 % (more common for infants and children). Mechanism of transmission is aerogenic (for exogenous infection), but disease can result from the spread of bacteria from the nasopharynx to the sinuses, blood, lungs, meninges, middle ear (endogenous infection).
- Prevention and immunity: immunity is type-specific; polyvalent conjugate or polysaccharide vaccines are recommended for all children aged 6 wk till 4–5 years, all adult ≥ 65 yr, and people of other ages with certain risk factors (chronic illnesses, immunosuppression, smokers, etc.).
- Because resistance to beta-lactams and macrolide antibiotics is increasing, seriously ill patients may be treated with an advanced-generation cephalosporins and respiratory fluoroquinolones.

MENINGOCOCCI

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Betaproteobacteria

Oder: Neisseriales
Family: Neisseriaceae
Genus: **Neisseria**
Species: *Neisseria meningitidis*

MORPHOLOGY

Gram-negative bean-shaped **diplococci;** havefimbriae (pili), nonmotile, nonsporing, possess *polysaccharide capsule*.

BIOLOGICAL PROPERTIRES

- Strict mesophiles: all media must be warmed to 37 °C prior to inoculation as the organism is extremely susceptible to temperature. This trait is rather unique among bacteria.
- Aerobes or facultative anaerobes, capnophiles.

MEDIA FOR CULTIVATION AND CULTURAL PROPERTIES

- Meningococci do not grow on simple media.
- Chocolate agar, blood agar, serum agar, Mueller-Hinton agar, Thayer-Martin medium (with antibiotics for inhibition of the nonpathogenic Neisseria and other contaminants).
- S-colonies are small, translucent, bluish grey (like "dew droops"); non-pigmented and nonhemolytic.

BIOCHEMICAL ACTIVITY

- Catalase and oxidase positive.
- Glucose and mannose are fermented, producing acid but no gas.

ANTIGENIC STRUCTURE

Based on capsular polysaccharide antigens (K-Ag) meningococci are classified into 13 or 14 serogroups. Group A is usually associated with epidemics; group C — mostly with local outbreaks, while group B causes both epidemics and outbreaks. Groups 29-E, W-135 and Y also frequently cause meningitis. Each serogroups can colonize nasopharynx, but these 6 serogroups (A, B, C, 29-E, W-135, Y) account for the large majority of meningococcal meningitis. Serogroups are further divided into serotypes based on **o**uter **m**embrane **p**roteins (OMPs).

ECOLOGY AND RESISTANCE

- Meningococci are obligate pathogens and facultative intracellular parasites of PMNs (leukocytes such as neutrophils).
- Meningococci are very delicate organisms, being highly susceptible to heat, desiccation, alterations of pH, and to disinfectants.
- They are sensitive to antibiotics but resistant strains have emerged and become common in many areas.

FACTORS OF PATHOGENICITY

1. Polysaccharide capsule (antiphagocytic).
2. Endotoxin is atypical and presents as **l**ipo**o**ligo**s**accharide (LOS), which has relatively short sugar chain than LPS and responsible for resistance to complement as well as the inflammatory tissue reaction.

3. Pili and OMPs are the major adhesins, which provide attachment of bacteria to the host mucosal cells.

CAUSED DISEASES

- Asymptomatic **nasopharyngeal microbocarriage** in 10–15 % of population.
- 10 % of carriers develop **acute nasopharyngitis**.
- In 1 % of carriers possible severe invasive infections — **acute cerebrospinal epidemic meningitis** and **meningococcemia** (meningococcal sepsis).

Meningococcal carriage and nasopharyngitis are the predominant forms of infection, being most spread in population. The term "*meningitis*" refers to inflammation the meninges of the brain or spinal cord. Meningitis usually begins suddenly (incubatory period 2–3 days), with intense headache, vomiting, stiff neck, photophobia and in some cases progresses to coma. Survivors may have sequelae such as permanent hearing loss, intellectual disability, etc. Maculopapular or hemorrhagic petechial rash often appears soon after disease onset. Fulminant meningococcemia includes septicemia, profound shock, cutaneous purpura, adrenal hemorrhage, multiple organ failure and **d**isseminated **i**ntravascular **c**oagulation syndrome (DIS-syndrome).

- **Meningococcal PSI** of lungs, joints, GU organs, eyes, endocardiumare less common.

EPIDEMIOLOGY

- Source of infection is sick persons and carriers: carriers are generally protected from meningitis and sepsis by antibodies but carrier state can transit to invasive diseases due to decrease of immunity (endogenous infection). Also a carrier is predominant source of exogenous infection for unprotected persons.
- Mechanism of transmission is airborne (rare close contact).

The incidence of epidemic meningitis is increased during winter and spring in temperate climates. Local outbreaks occur most frequently in sub-Saharan Africa between Senegal and Ethiopia, an area known as the **meningitis belt**. Meningococcal meningitis is common in children between 3 month and 5 years. Epidemics usually occur in semiclosed communities living in crowded conditions, as military recruits, college freshmen living in a dormitory, etc.

IMMUNITY, PREVENTION AND TREATMENT

- Immunity long-lasting, but group-specific.
- Meningococcal conjugate vaccine (consist of K-Ag of A, C, Y, W-135 serogroups); group B is almost not immunogenic (capsule consists of polyneuraminic acid which is not recognized by immune system). Vaccine is recommended for all children older than 2 years; also for groups of risk, laboratory workers, and travelers to endemic areas. Vaccination does not affect carrier status.
- Antibiotic therapy: penicillin G and third-generation cephalosporins. For chemoprophylaxis for those in close contact with diseased person (e.g., family) or for treatment of carriers must be used rifampicin instead of penicillin G.

GONOCOCCI

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Betaproteobacteria
Oder: Neisseriales
Family: Neisseriaceae
Genus: **Neisseria**
Species: *Neisseria gonorrhoeae*

MORPHOLOGY

Gram-negative bean-shaped **diplococci;** havefimbriae (pili). Pili undergo antigenic and phase variations. Unlike meningococci, *N.gonorrhoeae* cannot produce capsule.

BIOLOGICAL PROPERTIRES

- Strict mesophiles.
- Aerobes or facultative anaerobes, capnophiles.

MEDIA FOR CULTIVATION AND CULTURAL PROPERTIES

- Gonococci do not grow on simple media.
- Chocolate agar, blood agar, serum agar, Mueller-Hinton agar, Thayer-Martin medium.
- **S-colonies** are very small, opaque or transparent.

BIOCHEMICAL ACTIVITY

- Catalase and oxidase positive.
- Glucose is fermented, producing acid but no gas.

ANTIGENIC STRUCTURE

N.gonorrhoeae can capable to change their surface Ag to avoid immune response (marked antigenic variation). Gonococcal Ag includes: Ag of pili and Ag of outer membrane (OMPs). OMPs contain different proteins. Protein I (Por A and Por B) forms transmembrane channels (porins). Protein II is related to opacity of the gonococcal colonies (OPA protein). Protein III is Rmp-protein (reduction modifiable protein). Functions of OMPs: "perfect" adhesion of gonococci, inhibition of phagocytosis, induction of proinflammatory cytokine synthesis. More than 100 serotypes are known.

ECOLOGY AND RESISTANCE

• Gonococci are obligate pathogens and facultative intracellular parasites of PMNs (neutrophils).

• Gonococci are also very delicate organisms, being highly susceptible to factors of environment and to disinfectants.

• Gonococci producing β-lactamase have appeared and spread widely, these strains are known as **p**enicillinase-**p**roducing ***N.gonorrhoeae*** (PPNG). Gonococcal strains may be also resistant to tetracyclines and fluorquinolones.

FACTORS OF PATHOGENICITY

1. Fimbriae (pili) and OMPs for adhesion to the cells of urogenital tract); OMPs also inhibit phagocytosis.

2. IgA-protease (breakdown of sIgA).

3. Endotoxin (LOS) is responsible for resistance to complement as well as the inflammatory tissue reaction.

CAUSED DISEASES

❖ **Gonorrhea**

In men: predominant clinical form is *urethritis***;** in women — *urethritis* and *cervicitis*. Complications of genital infections in females include *pelvic inflammatory disease*(PID) which may lead to sterility and ectopic pregnancy.

Gonococci can also infect the rectum *(proctitis)* and oropharynx *(pharyngitis).* Rectal infections are common in women and homosexual men. Above described clinical forms are considered as acute gonorrhea but if not treated the disease easily becomes chronic.

In some rare cases the infection breaks tissue barriers, and bacteria emerge in the bloodstream (bacteremia). This leads to the disseminated gonococcal infections (DGI) such as *dermatitis* (skin rash), *arthritis, meningitis* or *endocarditis*.

About 50 % of women with cervical infections are asymptomatic. Asymptomatic carriage of gonococci is rare in men.

❖ **Blennophthalmia** (ophthalmia neonatorum, blennorrhoea)of newborns results from direct infection during passage through the birth canal. Gonococcal *conjunctivitis* also occurs in adults as a result of the transfer of the gonococci from the genitals to the eye.

EPIDEMIOLOGY

- Source of infection is sick persons and carriers (often women with asymptomatic infection).
- Mechanisms of transmission are sexual, rare contact, also contact during birth delivery; The infectivity of the organism is such that the chance of acquiring infection from a single exposure to an infected sexual partner is 20–30 % for men and even greater for women.

IMMUNITY, PREVENTION AND TREATMENT

- Protective immunity is not developing because of antigenic variety of gonococci. Repeated infections are common.
- There is no vaccine for prevention.
- Nonspecific prevention → condoms.
- The antibiotic of choice is penicillin G. Alternatives for use against penicillinase positive gonococci include third-generation cephalosporins and quinolones. Sex partner(s) should be referred and treated.
- For treatment of chronic gonorrhea multiple injections of gonococcal killed vaccine are administered to stimulate host immunity.
- To protect newborns against ophthalmia neonatorum, eye instillations with sodium sulfacyle, tetracycline or erythromycin solutions are applied immediately after birth.

Lecture 11. AEROBIC AND FACULTATIVELY ANAEROBIC GRAM-NEGATIVE RODS

BORDETELLA

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Betaproteobacteria
Oder: Burkholderiales
Family: Alcaligenaceae
Genus: **Bordetella**
Species: *Bordetella pertussis, B.parapertussis, B.bronchiseptica*

MORPHOLOGY

B.pertussis is small **Gram-negative** nonsporing, nonmotile **coccobacteria** with polypeptide capsule.

BIOLOGICAL PROPERTIES

- Mesophiles.
- Obligate aerobes, capnophiles.

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

- SR-variation of colonies during cultivation.
- Charcoal-blood agar → S-colonies with hemolysis.
- Bordet-Gengou medium (blood-potato-glycerin agar) → small opaque, greyish-white, retractile and glistening S-colonies, resembling "bisected pearls" or "mercury drops".

BIOCHEMICAL ACTIVITY

Biochemically Bordetella is relatively inert (does not ferment sugars). It produces oxidase and usually catalase.

ANTIGENIC STRUCTURE

Bordetella possesses somatic O-Ag and different surface antigens associated with capsular K-Ag (agglutinating factors). By agglutination test, 14 agglutinating factors have been identified. Factor 7 is common for all Bordetella species. Factor 1 is found only in strains of *B.pertussis*. Factor 14 is specific for *B.parapertussis* and factor 12 – for *B.bronchiseptica*.

ECOLOGY AND RESISTANCE

- *B.pertussis* is obligate human pathogen and extracellular parasite.
- It is delicate microorganism, being killed readily by heat, drying and disinfectants.

FACTORS OF PATHOGENICITY

1. Capsule (antiphagocytic).
2. Fimbriae (for adhesion).

3. Endotoxin (LPS).

4. **F**ilamentous **h**em**a**gglutinin (FHA) → promote adhesion of bordetella to the cilia of respiratory epithelium and to RBCs; also promote development of secondary infections with *Haemophilus influenzae* and *S.pneumoniae* by assisting their binding with respiratory epithelium ("piracy of adhesins"). FHA is used in acellular pertussis vaccine along with PT toxoid.

5. Exotoxins:

- *Pertussis toxin* (PT): it is believed to have an important role in pathogenesis of pertussis → spasms during cough and lymphocytosis. Expression of PT is unique to *B.pertussis*. PT can be toxoided; this toxoid is a major component of acellular pertussis vaccine (DTaP).
- *Dermonecrotic toxin* or heat-labile toxin → tissue damage in RT.
- *Tracheal cytotoxin* → destruction of tracheal cilia.
- *Adenylate cyclase toxin* → inhibition of phagocytosis.

6. Pertactin (PRN) is a highly immunogenic virulence factor of *B.pertussis*; it is OMP that promotes adhesion to tracheal epithelial cells. PRN is purified and is used for the acellular vaccine.

CAUSED DISEASES

B.pertussis is causative agent of 95 % of whooping cough (pertussis).

B.parapertussis is causative agent of 5 % of the parapertussis (similar to pertussis, but it is milder and less often fatal).

B.bronchiseptica is causative agent of acute respiratory infections resembling whooping cough (rarely infects healthy humans, though disease in immunocompromised patients has been reported).

Whooping cough is children respiratory infection. In adolescent and adults the diseases is often atypical and may present as bronchitis. Incubatory period 7–14 days.

Stages of whooping cough:

1. Catarrhal stage (1–2 weeks): low fever, rhinorrhea, malaise, sneezing and progressive cough (human is highly contagious).

2. Paroxysmal stage (4–8 weeks): violent and paroxysmal (spasmodic) cough that usually ends in a prolonged, high-pitched, crowing inspiration (characteristic "*whoop*"). Pressure effect during the attack of cough can provoke hemorrhage into brain, eyes, skin, and mucous membranes. In infants can be asphyxia.

3. Convalescent stage (recovery) (1–3 months): decline of the cough; secondary complications manifest as bronchopneumonia, otitis media, acute encephalopathy, seizures in infants, spastic paralysis, mental retardation and other neurological disorders.

EPIDEMIOLOGY

- Source of infection is sick persons and carriers.
- Mechanisms of transmission are aerogenic, rare direct contact.

IMMUNITY, PREVENTION AND TREATMENT

- Long-lasting immunity.

• Vaccination: DPT or pertussis killed vaccine (three injections in intervals of 4–6 weeks are to be given before the age of 6 months, followed by booster dose at the end of first year of life). Acellular vaccine (DTaP) includes PT, FHA and PRN. Use of acellular vaccines is recommended in preference to the whole cell vaccine because of decreased side effects.

• Antibiotic therapy is beneficial only if initiated within first 10 days of the disease (drug of choice is erythromycin).

HAEMOPHILUS

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Pasteurellales
Family: Pasteurellaceae
Genus: **Haemophilus**
Species: *Haemophilus influenzae, H.parainfluenzae, H.ducreyi*

MORPHOLOGY

Gram-negative coccobacteria is nonmotile, nonsporing and often capsulated. Pleomorphism is characteristic (in sputum — clusters of coccobacteria, in CSF — long and filamentous forms are predominant).

BIOLOGICAL PROPERTIES

• Facultative aerobes
• Methophiles

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

• Fastidious growth requirements. They require one or both of two **growth factors** which are present in blood and essential for growth:

► **X factor** is hemin of RBCs;

► **V factor** is coenzyme NAD or NADP (present in erythrocytes and in many other animal cells, it synthesized in excess by some fungi and bacteria like *S.aureus* and released in surrounding media).

• **Satellitism** is phenomenon when haemophilus colonies are large near growth of staphylococcus and smaller away from it.

• Chocolate agar is best medium for cultivation.

BIOCHEMICAL ACTIVITY

Catalase and oxidase are positive.

ANTIGENIC STRUCTURE

• O-Ag.

• K-Ag: on base of capsular polysaccharide *H.influenzae* strains have been classified into 6 serotypes (a-f). Most virulent type b (*H.influenzae type b* — **Hib**) because it has unique chemical structure (contains pentose sugars ribose and ribitol instead of hexoses).

• *H.influenzae* strains lacking capsule are called "nontypable" (next to Hib these strains are the most relevant in clinical infections).

ECOLOGY AND RESISTANCE

• *H.influenzae* is opportunistic pathogen and extracellular parasite of URT of 30–50 % of healthy people. Other species are normal flora (e.g., *H.parainfluenzae*).

• Haemophilus is very delicate bacterium and not resistant in nature.

FACTORS OF PATHOGENICITY

1. Polysaccharide capsule (for Hib — polyribitol phosphate capsule) is the most important virulence factor (antiphagocytic effect);
2. Fimbria (for adhesion);
3. Endotoxin (LPS);
4. Exotoxins (cytotoxins, hemolysins);
5. IgA-protease.

CAUSED DISEASES

H.influenzae and *H.parainfluenzae* produce **PSI**.

H.ducreyi → **chancroid** of genitalia ("soft chancre") is venereal disease.

Diseases caused by *H.influenzae* may be considered fewer than two groups — invasive and noninvasive.

Invasive infections → bacteria spread by blood, being protected from phagocytosis with capsules. The most important infection is hemophilic **meningitis**, others – **epiglottitis**, **bacteremia, pneumonia, endocarditis, arthritis**. These infections are usually seen in children and caused by Hib.

Noninvasive infections → bacilli spread by local invasion along mucosal surfaces and cause secondary infections, usually of respiratory tract (**otitis media, sinusitis, chronic bronchitis**). They are usually seen in adults and caused by noncapsulated strains.

EPIDEMIOLOGY

• Source of infection is sick persons and carriers. *H.influenzae* colonizes healthy children and adults (although the rate of colonization is far greater for nontypable strains than for Hib).

• Mechanisms of transmission are aerogenic and direct contact.

IMMUNITY, PREVENTION AND TREATMENT

• Immunity is type specific.

• Hib conjugate vaccine is available for children ≥ 2 mo of age and has reduced invasive infections.

• Antibiotic choice requires susceptibility testing.

LEGIONELLA

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Legionellales

Family: Legionellaceae	Family: Coxiellaceae
Genus: **Legionella**	Genus: **Coxiella**
	Species:*Coxiella burnetii*
Species:*Legionella pneumophila*	

MORPHOLOGY, BIOLOGICAL AND CULTURAL PROPERTIES

- **Gram-negative** pleomorphic aerobic **rods** which are motile, nonsporing and non-capsulated. Long, filamentous forms may develop, particularly after growth on the surface of agar.
- Legionella grows only on special media with L-cysteine and iron in atmosphere containing 5 % of CO_2 (charcoal yeast extract agar).
- They can ferment sugars, catalase are positive, produce S-colonies.

ECOLOGY, ANTIGENIC STRUCTURE AND FACTORS OF PATHOGENICITY

- Soil and damp biotopes are the natural reservoirs of Legionella (saprophytes). These organisms are facultative intracellular parasites of alveolar macrophages.
- Pathogenic factors: factors of adhesion and invasion, OMPs for inhibition of phagocytosis, endotoxin, secretory protein – cytolysin with toxic and antiphagocytic activity.
- *L.pneumophila* includes 12 serogroups; human infections are caused mainly by serogroup 1.

CAUSED DISEASES

Two clinical forms of **legionellosis** have been described:

► **Legionnaire's disease**: clinical picture is characterized by acute pneumonia which varies in severity from mild illness ("walking pneumonia") to fatal pneumonia. Typically, patients have high fever and cough. Extrapulmonary symptoms, such as headache, confusion, muscle aches, and gastrointestinal disturbances, are common. Occurrence is more likely for persons with immunodeficiencies and cardiopulmonary diseases, also smokers over 55 years with high alcohol intake.

► **Pontiac fever**: catarrhal symptoms, including fever, headache, and severe muscle aches (self-limited, and convalescence is uneventful).

► **Extrapulmonary PSI** (e.g., pericarditis and endocarditis) are rare.

EPIDEMIOLOGY

Legionellosis occurs in epidemic form or in sporadic cases.

- Sources of infection are hot and cold water supply systems, cooling towers, air moisturizing units in air conditioners, swimming pools, domestic water systems and showers, ice making machines, fountains, whirlpool baths, etc.
- Transmission is airborne via respiratory droplets containing the bacteria — aerosols (human-to-human transmission has not been confirmed).

TREATMENT AND PREVENTION

- Macrolides are antibiotics of choice.

• Decontamination of identified environmental sources. Legionella resist water temperature as high as 50 °C. Above 70 °C Legionella dies almost instantly.

Coxiella

• **Gram-negative** pleomorphic small **coccobacteria**.

• Traditionally coxiella was classified with rickettsia.

• Obligate intracellular parasite and obligate pathogen.

• Very resistant in nature and to disinfectants.

• There two variants of *C.burnetii* based on LPS structure. The virulent natural form ("phase I") has "smooth" LPS while "phase II" — avirulent laboratory strain — has "rough" LPS.

• *C.burnetii* has complex life cycle (SCV — "small cell variant" and LCV — "large cell variant"). SCV is metabolically inactive, extracellular, resistant form. After inhalation SCV infects alveolar macrophages, activates and transforms into LCV, which is intracellular active form for binary fission. After multiplication LCV is transforming again into SCV and release by exocytosis.

• **Q-fever** varies widely in severity, including asymptomatic, acute or chronic *febrile disease, granulomatous liver disease,* and chronic infection of the heart valves *(endocarditis).C.burnetii* proliferates in the lungs, causing *atypical pneumonia* in some patients. Patients with immunosuppression and pregnant women have a worse prognosis.

• Q-fever (coxiellosis) is zoonotic disease (domestic animals, birds, ticks). Human Q-fever follows inhalation of aerosol particles derived from heavily infected placentas of sheep, goats, cattle, and other mammals; also after contact with animals or contaminated dung and bedding, during consumption of raw eggs, raw milk. Unlike Rickettsia Coxiella is not transmitted in humans via tick bites. Tick vector is involved in spread of the bacteria among the reservoir animal hosts. Human-to-human transmission is extremely rare.

• Q-fever is considered to be an occupational hazard for farmers, veterinarians, abattoir workers, and laboratory personnel. Disease has been reported in almost every country, except for New Zealand.

• Antibiotic therapy is recommended only for severe cases of Q-fever. Human and animals vaccines are made form killed bacteria of phase I.

• *C.burnetii* is also significant in biological warfare due to its high infectivity in low doses, aerosol exposure route, environmental resistance, rapid onset of the disease and significant morbidity.

NONFERMENTING BACTERIA (NFB)

PSEUDOMONAS

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria

Order: Pseudomonadales
Family: Pseudomonadaceae
Genus: **Pseudomonas**
Species: *Pseudomonas aeruginosa*

MORPHOLOGY

Gram-negative slender **rods**, motile by polar flagella. It is noncapsulated but many strains have mucoid slime layer.

BIOLOGICAL PROPERTIES

• Obligate aerobes.
• Mesophiles, but can grow at a wide range of temperatures (6–42 °C)

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

• *P.aeruginosa* grows on simple media (MPA, MPB), blood agar (hemolytic colonies), DDM (Endo, EMB, MacConkey agar → lactose-negative colonies).
• Large, opaque, irregular S-colonies with a distinctive, sweet or fruity smell; in broth, it forms a dense turbidity with a surface pellicle.
• *P.aeruginosa* produces a number of pigments, the best known being **pyocyanin** (colonies are bluish green and characteristic "blue pus") and fluorescin. Some strains may be nonpigmented.

BIOCHEMICAL ACTIVITY AND ANTIGENIC STRUCTURE

• Aerobic **nonfermenter** (metabolism is oxidative); glucose is utilized oxidatively, forming only acid.
• Catalase and oxidase are positive.
• O-Ag.

ECOLOGY AND RESISTANCE

• Pseudomonas is ubiquitous, mostly saprophytic, being found in water, soil or other moist environments. Extremely adaptive organisms which can survive and multiply even with minimal nutrients if moisture is available (e.g., sinks, respirators, endoscopes, bedpans, lotions, ointments, eye drops, distilled water, antiseptic solutions, flowers, etc.)
• Some of them are pathogenic to plants, insects and reptiles. A few cause human opportunistic infections.
• *P.aeruginosa* can present in axilla and perineum of some healthy persons, fecal carriage is common after antibiotic therapy.
• Resistant to common antiseptics and disinfectants.
• *P.aeruginosa* possesses a **natural resistance** to many antibiotics.

FACTORS OF PATHOGENICITY

1. Fimbriae (for adhesion).
2. Endotoxin (LPS).
3. Slime layer (act as a capsule and formation of biofilms).
4. Exotoxins (exotoxins A and S, hemolysins, enterotoxins).
5. Invasion enzymes (proteases, lipases, elastases, etc.).

CAUSED DISEASES

P.aeruginosa produces different opportunistic **PSI**:

Common infection outside of hospitals is suppurative **otitis**. In hospital it may cause following clinical forms:

- **Infections of wounds, bedsores, burns** (commonly results in a generalization and fatal **sepsis**).
- **UTI** (following installation of catheters or urological manipulations).
- **Meningitis (**following lumbar puncture).
- **Endocarditis**(following cardiac surgery).
- Post-tracheostomy and ventilator-associated necrotizing **pneumonia**.
- Severe **corneal infections**(following eye surgery or injury).
- Infantile **diarrhea** and **sepsis**.

Most *cystic fibrosis* patients are chronically colonized with *P.aeruginos*a.

EPIDEMIOLOGY

- Source of infection is sick persons and carriers; different environment areas (water, toilets).
- Mechanisms of transmission are aerogenic, contact (with medical instruments, respirators, endoscopes, flowers, eye drops, etc.).

P.aeruginosa is important **hospital-acquired pathogen**, especially for ventilator patients, burns patients and patients with immunosuppression.

IMMUNITY, PREVENTION AND TREATMENT

• Immunity is type specific.

• Topical therapy of burn wounds with antibacterial agents such as silver sulfadiazine, coupled with surgical debridement, has dramatically reduced the incidence of *P.aeruginosa* sepsis in burn patients. No flowers or raw vegetables in burn units.

• Strict attention to asepsis. Careful cleaning and monitoring of respirators, catheters and other medical instruments.

• Various antibiotics depending on site of infection and susceptibility testing. *P.aeruginosa* is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to gentamicin, tobramycin, colistin and amikacin, resistant forms have developed.

Acinetobacter

- *Acinetobacter baumannii* is aerobic, nonfermentative **Gram-negative rods** that are resistant to most antibiotics.
- *A.baumannii* forms opportunistic infections (severe pneumonia, tracheobronchitis, UTI, sepsis, abscesses in any organ, etc.).
- **M**ulti**d**rug-**r**esistant ***A.b****aumannii* (MDR-AB) has always been inherently resistant to multiple antibiotics.
- Acinetobacter enters into the body through open wounds, catheters, and breathing tubes. It usually infects those with compromised immune systems, such as the wounded, the elderly, children, or those with immune diseases.

Stenotrophomonas

• *Stenotrophomonas maltophilia* is aerobic, nonfermentative, **Gram-negative rods**.

• *S.maltophilia* is ubiquitous in aqueous environments, soil and plants. It frequently colonizes breathing tubes such as endotracheal or tracheostomy tubes and urinary catheters.

• In immunocompetent individuals, *S.maltophilia* is a relatively unusual cause of pneumonia, UTI or sepsis. Stenotrophomonas infections have been associated with high mortality in immunocompromised persons.

• *S.maltophilia* is naturally resistant to many broad-spectrum antibiotics and is thus often difficult to eradicate.

Burkholderia

• Burkholderia is aerobic, nonfermentative, **Gram-negative rods**.

• *B. cepacia* is opportunistic pathogen, particularly in those with cystic fibrosis or chronic granulomatous diseases, in whom it causes necrotizing fatal pneumonia. Also it can cause UTI, respiratory and wound infections, peritonitis, endocarditis and sepsis. *B. cepacia* inherently resistant to most antibiotics.

• *B. mallei* is causative agent of **glanders** (high-risk zoonotic infection).

• *B. pseudomallei* is causative agent of **melioidosis** (glanders-like high-risk infection).

Lecture 12 ENTEROBACTERIA

GENERAL CHARACTERISTICS OF ENTEROBACTERIA

1. Enterobacteria are **Gram negative rods**, nonsporing, may or may not be capsulated and are motile (peritrichous) or nonmotile.

2. Mesophiles, facultative anaerobes, grow on simple media, ferment glucose producing acid and gas or acid only (fermentation of other sugars varies). The four biochemical tests are employed in the classification of Enterobacteria: *indole production*, *methyl red*, *Voges-Proskauer* and *citrate utilization* tests (they are generally referred to the mnemonic "IMViC"). Also Enterobacteria are catalase positive and oxidase negative.

3. The oldest method was to classify these bacteria into two groups based on their lactose fermentation on DDM (Endo, MacConkey, Ploskirev, EMB agars). **Nonlactose fermenters** produce colorless colonies, while **lactose fermenters** – colored colonies (due to the indicator in media). Lactose fermenters are major intestinal commensals (Escherichia, Klebsiella and Enterobacter). Nonlactose fermenters are major intestinal pathogens (Salmonella, Shigella and Yersinia).

4. Media for accumulation of pure culture and its biochemical identification is **t**riple **s**ugar **i**ron agar (TSI agar, which consist of slant MPA, indicator, glucose, lactose, sucrose, reagents for urease and H_2S production) or **K**ligler **i**ron agar (KI agar, which consist of slant MPA, indicator, glucose, lactose, reagent for H_2S production).

5. The modern taxonomy is based on common morphological, biochemical properties and similar DNA base composition (table 15).

Table 15 — Modern classification of Enterobacteria

Family: **Enterobacteriaceae**				
Tribe I: **Escherichiae**	Tribe II: **Klebsiellae**	Tribe III: **Proteae**	Tribe IV: **Erwiniae**	Tribe V: **Yersiniae** Genus Yersinia
Genera: 1. Escherichia 2. Edwardsiella 3. Citrobacter 4. Salmonella 5. Shigella	Genera: 1. Klebsiella 2. Enterobacter 3. Hafnia 4. Serratia	Genera: 1. Proteus 2. Morganella 3. Providencia	Genus: Erwinia	
The genus includes species which are classified into different types: biotypes, serotypes and phagotypes				

6. Natural habitat of Enterobacteria is the intestinal tract of humans and animals. Some species cause the characteristic diseases (such as typhoid fever, dysentery), as well as many opportunists that cause PSI, including nosocomial infections.

7. The most important pathogenic factors are fimbriae, invasins, endotoxin (LPS) and various exotoxins.

Classification of exotoxins of Enterobacteria:

■ **LT** (heat **l**abile entero**t**oxin) activates adenyl cyclase in the enterocytes to form cAMP, leading to increased outflow of water and electrolytes into the gut lumen resulting in diarrhea.

■ **ST** (heat **s**table entero**t**oxin) acts by activation of cGMP in the intestine resulting in diarrhea.

■ **Shiga toxin** (verotoxin) and **Shiga-like toxin** (SLT) are cytotoxins.Shiga toxin is produced by *Shigella dysenteriae*. Shiga-like toxinis produced by some *E.coli* group (EHEC). Shiga toxin and SLT act by inhibition of protein synthesis within the target cells (renal epithelial cells, neurons and endothelium) resulting in hemorrhages, necrosis and development of ***hemolytic uremic syndrome*** (HUS).

ESCHERIHIA

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: **Escherichia**
Species: *Escherichia coli*

MORPHOLOGY

Gram-negative motile (peritrichous), straight **rods** with rounded ends. Microcapsules are found in the most of strains. Sporesare not formed.

BIOLOGICAL PROPERTIRES

- Facultative anaerobes.
- Mesophiles.

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

- *E.coli* can grow on simple media (MPA, MPB).
- S-colonies are large with greyish white appearance (SR-variation occurs as result of subculturing).
- DDM (Endo agar, EMB-agar) → lactose-positive colonies (for commensal serogroups), for pathogenic groups (EHEC, EIEC) → lactose-negative colonies.
- Blood agar → hemolytic colonies.
- In broth growth occurs as general turbidity with heavy deposit.

BIOCHEMICAL ACTIVITY

- Glucose, maltose, lactose and other sugars are fermented with the production of acid and gas.
- Indole positive.
- *E.coli* is lactose-fermenter (exception of some pathogenic serogroups such as EHEC, EIEC, etc.).

ANTIGENIC STRUCTURE

Serotyping of *E.coli* is based on Ag: **O-Ag**, **K-Ag** and **H-Ag**.

- 170 types of O-Ag, 100 types of K-Ag and 75 types of H-Ag have been recognized.

• The *antigenic formula* of a strain is recorded as a number of the particular antigens. Serogroup is characterized by O-Ag (or OK-Ag). Respectively, there are 170 serogroups of *E.coli*. Further combination of O-Ag (OK-Ag) and H-Ag is corresponding to serotype (e.g., O111 is serogroup; O111**:**K58**:**H2 is serotype). The various combinations of antigens result in >1000 known serotypes of *E.coli*.

• Specific serotypes are associated with certain diseases. The normal colon strains (commensal strains) generally belong to "early" O-serogroups (e.g., O1, O2, O3), while the enteropathogenic strains belong to "later" O-serogroups (O26, O55, O111, O157, etc.).

ECOLOGY AND RESISTANCE

• The natural habitat of *E.coli* is the intestinal tract of humans and animals (colon normal flora).

• Distributive and relatively resistant in nature (*E.coli* is anindicator organism of fecal contamination of water and foods);

• Most of *E.coli*serogroups belong to opportunistic pathogens (commensals of colon), while some serogroups consider as obligate pathogens (e.g., EPEC, ETEC, EHEC and EIEC).

FACTORS OF PATHOGENICITY

1. Fimbriae, microcapsule (for adhesion).
2. Endotoxin (LPS).
3. Extracellular enzymes and OMPs (for invasion).
4. Exotoxins: enterotoxins (LT, ST), cytotoxins (SLT for EHEC).

CAUSED DISEASES

There are several clinical forms of **escherichioses**: parenteral infections (PSI of different localization; caused by commensal or nonenteropathogenic *E.coli* groups such as UPEC, SEPEC and MENEC); human-to-human transmission is absent; autoinfections; predisposing factors are suppression of immunity and medical interventions) and enteral or coli-infections (diarrheal infections; caused by diarrheagenic*E.coli* groups with fecalo-oral transmission).

❖ **PSI, including nosocomial**: UTI (cystitis, urethritis, pyelonephritis, asymptomatic bacteriuria), sepsis, neonatal meningitis, peritonitis, appendicitis, pneumonia, etc. UTI is caused by UPEC (uropathogenic *E.coli*), sepsis – by SEPEC (sepsis-associated *E.coli*), and neonatal meningitis — by MENEC (meningitis-associated *E.coli*).

❖ **Diarrheal infections**: diarrheagenic *E.coli* groups are: enteropathogenic *E.coli* (EPEC), enterotoxigenic *E.coli* (ETEC), enteroinvasive *E.coli* (EIEC), enterohemorrhagic (verotoxigenic) *E.coli* (EHEC) and enteroaggregative *E.coli* (EAggEC).

Table 16 — Pathogenic *E.coli* groups and their characteristics

Pathogenic group	Factors of pathogenicity	Associated diseases
EPEC → small intestine (O26, O55, O111)	● Adhesins (bundle-forming pili) and OMP (intimin) ● Enterotoxins are absent ● Specific pathogenesis* ● Endotoxin (LPS)	Watery diarrhea of infants (less than 1 year)
ETEC → small intestine (O8, O15, O25, O27)	● Adhesins ● LT and/or ST ● Endotoxin (LPS)	Cholera-like escherichiosis, diarrhea of travelers
EIEC → colon (O124, O152, O154, O164)	● Enterotoxins are absent ● Highly invasive ● Endotoxin (LPS)	Dysentery-like escherichiosis
EHEC → colon (O 26, O157, O11; the most important serotype is O157**:**H7)	● Adhesins and "effacement" of enterocytes ● **SLT** ● Endotoxin (LPS)	Hemorrhagic colitis with HUS (hemolytic uremic syndrome)
EAggEC → small intestine	● AAF (aggregative adhesion fimbria) ● Specific cytotoxins ● Endotoxin (LPS)	Diarrhea of travelers, diarrhea of HIV-positives, endemic diarrhea
UPEC →urinary tracts (O1, O2, O6, O18, O75)	● Mannose-sensitive and mannose-resistant fimbria ● P-fimbria ● Specific cytotoxins ● Endotoxin (LPS)	UTI (cystitis, pyelonephritis)
SEPEC →blood	● Fimbria ● Endotoxin (LPS) ● Resistance to opsonins and phagocytic factors ● Damage of endothelium	Bacteremia and sepsis (septicemia)
MENEC →brain	Resemble factors of SEPEC	Neonatal meningitis

***** Pathogenesis of watery diarrhea includes stages: 1) adhesion of bacteria and "effacement" of enterocytes (following by formation of secretory complex of III type and transfer of the "injective" bacterial toxins into the enterocytes); 2) damage of cytoskeleton in the enterocytes and microvilli; 3) development of diarrhea.

EPIDEMIOLOGY

• Source of infection is exogenous (human and animals) and endogenous (patient's own colonic flora).

• Mechanisms of transmission are autoinfection, contact with infected birth canal of mother, fecalo-oral. For example, EHEC-infection is zoonosis (cattle), transmission is fecalo-oral (with undercooked meat); UPEC-infection is autoinfection, etc.

IMMUNITY, PREVENTION AND TREATMENT

• Nonlong-lasting immunity.

• Necessary sanitary control of water and foodstuff contamination.

• No vaccination.

• Severe diarrhea requires oral replacement of fluid and electrolyte losses. Antibiotic therapy is used for PSI. Probiotics are beneficial.

SHIGELLA

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: **Shigella**
Species: *Shigella dysenteriae,S.flexneri, S.boydii, S.sonnei*

MORPHOLOGY

Gram-negative nonmotile, nonsporing, noncapsulated straight **rods**.

BIOLOGICAL PROPERTIRES

- Facultative anaerobes
- Mesophiles

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

- Can grow on simple media (MPA, MPB).
- S-colonies are small, convex and translucent.
- DDM (Endo, EMB, MacConkey agar) → lactose-negative colorless colonies (exception is *S.sonnei* which ferments lactose late and forms lactose-positive pink colonies).
- SS-agar(**s**almonella-**s**higella agar) is selective medium.

BIOCHEMICAL ACTIVITY

- Glucose is fermented with production of acid without gas except for the some biotypes of *S.flexneri* of serotype 6, which also form gas.
- Mannitol fermentationis of importance for shigella biochemical classification (there are mannitol-nonfermentating species like *S.dysenteriae* and others — mannitol-fermentating species);
- Lactose is not fermented except of *S.sonnei* which ferments it late.

ANTIGENIC STRUCTURE

- Somatic O-Ag.
- Capsular K-Ag.
- Species includes different number of serotypes based on O-Ag: *S.dysenteriae* (13 serotypes), *S.flexneri* (15 serotypes), *S.boydii* (18 serotypes), *S.sonnei* (1 serotype).

ECOLOGY AND RESISTANCE

- Shigella is obligate enteric pathogen and facultative intercellular parasite.
- Shigella is not highly resistant in nature.

FACTORS OF PATHOGENICITY

1. Fimbriae (for adhesion).
2. Endotoxin (LPS).
3. Extracellular enzymes and OMPs (for invasion).

4. Exotoxins:

• Shiga toxin (produced by *S.dysenteriae* type 1, three activities: neurotoxic, cytotoxic and enterotoxic; responsible for HUS).

• Other species of shigella can produce ST and/or LT.

♦ Fundamental event in pathogenesis of Shigella is its ability to invade and colonize the human intestinal epithelium. The pathogenesis is a multistep process which depends on the capacity of the bacteria to cross the colonic mucosa via M-cells associated with intestinal lymphoid tissue (this capacity is encoded by virulence plasmids). Shigella uses a **type III secretion system**, which acts as a "biological syringe" (injectisome) to transfer toxic effector proteins to the target cells. The effector proteins can induce actin polymerization to facilitate intracellular motility of bacteria inside the host cells, helping in cell-to-cell spread. After invasion, Shigella multiplies intracellularly and spread to neighboring epithelial cells, resulting in tissue destruction.

CAUSED DISEASES

Shigellosis (**bacillary dysentery**) has clinical symptoms: fever, dehydration, bloody diarrhea (*melena*); inflammation and ulceration of large intestine (shallow ulcers), abdominal pain, and death from kidney failure. Intermittent painful rectal spasm (tenesmus) is characteristic for developed shigellosis. Incubation period lasts from one to several days. Prevalent clinical form of shigellosis is *hemorrhagic enterocolitis*, also possible *gastroenterocolitis, gastroenteritis*. Severity depends on age of patient and virulence of a strain. *S.dysenteriae* type 1 is the most virulent agent of dysentery.

EPIDEMIOLOGY

- Source of infection is sick persons and carriers.
- Mechanism of transmission is fecalo-oral.

Shigellosis occurs in areas of poor sanitation. Lack of personal and domestic hygiene is important in contributing to the spread of infection. *S.dysenteriae* accounts for deadly epidemics in developing countries, *S.flexneri* and *S.sonnei* are endemic for developing countries and can be found in developed ones. *S.boydii* accounts for most of cases in India.

IMMUNITY, PREVENTION AND TREATMENT

- Nonlong-lasting immunity. Repeated infections are possible.
- No vaccination. Polyvalent bacteriophages can be used for prophylaxis.
- Antibiotic and probiotic therapy is useful. Urgent infusion therapy to compensate water and electrolyte loss is used.
- Prevention: proper sanitation (sewage, hand washing, cleaning of drinking water, detection and treatment of carriers, etc.).

SALMONELLA

TAXONOMY

Domain: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: **Salmonella**
Species: *Salmonella enterica*, *S.bongori*

S.enterica includes several subspecies: arizonae, diarizonae, houtenae, salamae, indica and enterica (ssp. enterica is predominant).

S.entericassp. enterica includes many typhoidal and nontyphoidal serotypes (generally ≥ 2500): *S.Typhi*, *S.Paratyphi*, *S.Enteritidis*, etc.

MORPHOLOGY

Gram-negative motile, nonsporing, noncapsulated straight **rods**.

BIOLOGICAL PROPERTIRES

- Facultative anaerobes.
- Mesophiles.

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

- Salmonella can grow on simple media (MPA, MPB).
- S-colonies are large, greyish and translucent.
- Bile broth, selenite F broth, bile broth are enrichment media.
- Wilson-Blair bismuth sulfite agar (differential-selective medium) → black colonies due to H_2S production.
- DDM (Endo agar, EMB-agar) → lactose-negative colonies.
- MacConkey agar and SS-agarare selective media.

BIOCHEMICAL ACTIVITY

- Salmonella ferments glucose, mannitol and maltose, forming acid and gas (exception *S.Typhi* which doesn't produce gas).
- Lactose is not fermented.
- $H_2S$is produced except of *S.Paratyphi A.*

ANTIGENIC STRUCTURE

- Somatic O-Ag.
- Flagella H-Ag.
- Capsular Vi-Ag.

Kauffman-White classification (table 17) is a system that classifies the genus Salmonella into serogroups and serotypes, based on surface Ag. Salmonella is classified into 67 serogroups based on the presence of characteristic O-Ag ("**group factor**"). There are five "main groups" that responsible for the most of salmonella infections (A, B, C, D and E). Other groups are collectively called "rare groups". Salmonella can exist in two phases; motile and nonmotile (it is called "**phase variation**"). Different H-Ag are produced depending on the phase in which salmonella is found. *Monophasic* and *diphasic* serotypes have been recognized. Several strains (ex.: *S.Typhi*) carry an additional microcapsular Ag, **"Vi-Ag"**, so-called because of the enhanced **vi**rulence of a strain.

Kauffman-White classification is using for serological identification (serotyping) of pure culture with help of slide agglutination reaction.

For example, for serological identification (serotyping) of *S.Typhi* pure culture have been used: O9-antiserum, H(d)-antiserum and Vi-antiserum because of the antigenic formula of *S.Typhi* (serotype) is O9**:**H(d)**:**Vi.

Table 17 — Kauffman-White classification of salmonella (fragment)

Sero-groups	**Serotypes**	**Group factor (O-Ag)**	**Phase 1 H-Ag** (motile)	**Phase 2 H-Ag** (non-motile)	**Vi-Ag**
A	*S.Paratyphi A*	O–2	a	-	-
B	*S.Paratyphi B*	O–4	b	1, 2	-
	S.Typhimurium		i	1, 2	-
C	*S. Paratyphi C*	O–6, O–7, O–8	c	1, 5	+
D	*S.Typhi*	O–9	d	-	+
	S.Enteritidis		g, m	-	-
E	*S.Oxford* *S.Zanzibar*	O–-3	a	1, 7	-
			k	1, 5	-

ECOLOGY AND RESISTANCE

- Salmonella is obligate enteric pathogen and facultative intracellular parasites of monocytes/macrophages.
- Salmonella is "problematic bacteria" and can develop **drug resistance** (multiresistant salmonella); such strains are responsible for intrahospital outbreaks.
- Salmonella possess a marked resistance in the environment (in soil, dairy products, meat, fruits, etc.).

FACTORS OF PATHOGENICITY

1. Fimbriae (for adhesion).
2. Endotoxin (LPS): potent virulent factor of salmonella; it activates macrophages and provokes inflammatory cytokines release that leads to tissue damage and severe intoxication (affects CNS).
3. Extracellular enzymes (for invasion).
4. Enterotoxins: LT, ST.
5. Vi-Ag (protection against phagocytosis and complement activation).

Similar with other invasive bacteria (Shigella, Yersinia, EPEC and EHEC), Salmonella possess **type III secretion system** with bacterial "needle" complex (injectisome) by which salmonella transfers effector proteins into M-cells of jejunum causing change of cellular cytoskeleton with subsequent invasion by endocytosis. Bacteria invade the host cells and then spread into the lymphatic follicles and Peyer's patches (invasion of mesenteric lymph nodes is present during incubatory period of typhoid). Further bacteria spread to the bloodstream and clinical symptoms are appearing (1^{st} wk of typhoid).

♦ *S.Typhi* penetrates gastrointestinal epithelium and disseminates with blood (→ bacteremia). Nontyphoidal strains usually remain within GIT where

they cause local inflammation (→ enteral salmonellosis). Manifest illness usually begins from diarrhea and vomiting. Due to possible survival of salmonella in the epithelial cells and macrophages generalization of the infection can be developing in the immunocompromised patients and newborns (→ parenteral salmonellosis). Very often parenteral salmonellosis is caused by multiple antibiotic-resistant strains of *S.Typhimurium* and finally results in sepsis.

CAUSED DISEASES

Salmonella causes the fallowing diseases: **typhoid**; **paratyphoidA, B** and**C**, **enteral salmonellosis** (gastroenteritis or food poisoning) or**parenteral salmonellosis** (PSI).

Table 18 — Characteristics of salmonella diseases

Serotype	Disease	Clinical picture	Epidemiology
S.entericaserotype ***Typhi***	**Typhoid** ("enteric fever")	Symptoms depend on stage of disease (table 19)	SI*: sick persons and carriers MT: fecalo-oral
*S.enterica*serotypes ***Paratyphi A, B, C***	**Paratyphoid**	Paratyphoid has similarities with typhoid fever, but its course is more benign	SI: sick persons and carriers for paratyphoid A, animals for paratyphoid B, C MT: fecalo-oral
*S.enterica*serotypes ***Typhimurium, Enteritidis,*** *etc.*	**Salmonellosis** (frequent nosocomial infection)	Enteral form (gastroenteritis)	SI: animals MT: fecalo-oral
		Parenteral forms (sepsis, osteomyelitis, meningitis, pneumonia, etc.)	SI: sick persons and carriers MT: fecalo-oral, contact

*SI — source of infection; MT — mechanisms of transmission.

Table 19 — Clinical stages and laboratory diagnosis of typhoid

Stage	Pathogenesis stage	Clinical picture	Laboratory diagnosis
1 week	Bacteremia	High fever, bradycardia, malaise, headache. To end of 1st wk **delirium** is frequent ("status typhosus")	Bacteriological method (hemoculture)
2 week	Diffusion of Salmonella into parenchymatous organs (kidney, spleen, liver)	Roseolar spots (on chest, abdomen), diarrhea or constipation, hepatosplenomegaly	Serodiagnostics (tube agglutination test or Widal reaction, ELISA)
3 week (critical stage)	2nd invasion of mesenteric lymph nodes - development of DTH → necrosis of Peyer's patches	Complications can occur: intestinal hemorrhage, perforation in the ileum, neuropsychiatric symptoms (coma), abscesses, pneumonia, etc.	Bacteriological method (bili-, urino- and coprocultures) Serodiagnostics
4 week	Risk of microbocarriage Reconvalescence	Storage of salmonella in liver, gall bladder	Bacteriological method Serodiagnostics

IMMUNITY, PREVENTION AND TREATMENT

- Long-lasting immunity after typhoid (but possible relapse and microbocarriage). Salmonellosis → weak immunity.
- There are two vaccines for use for the prevention of typhoid: live oral vaccine and injectable Vi polysaccharide vaccine. Both are recommended for travelers to areas where typhoid is endemic.
- Sanitation and hygiene are the critical measures that can be taken to prevent typhoid. Careful food preparation and washing of hands are crucial to preventing typhoid.
- Antibiotic therapy of typhoid: aminopenicillins, fluorquinolones. In case of salmonellosis to take into account multidrug resistance of some serotypes; for symptomatic therapy can be used replacement of water and electrolytes.

INTESTINAL YERSINIA

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: **Yersinia**
Species: *Yersinia enterocolitica*, *Y.pseudotuberculosis*

MORPHOLOGY

Gram-negative, nonsporing, short, ovoid **rods** (coccobacteria) with bipolar staining, arranged singly or in small groups. Bacteria are motile at room temperature (20–28 °C) and non-motile at 36–37 °C. It is nonsporing and capsulated. Pleomorphism is very common.

BIOLOGICAL PROPERTIRES

- Facultative anaerobes.
- Psychrophilic (optimal temperature is 28–29 °C).

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

- Intestinal yersinia can grow on simple media (MPA, MPB).
- S-colonies are small and transparent. SR-dissociation is possible (especially for *Y.pseudotuberculosis*).
- DDM (Endo agar, EMB-agar) → lactose-negative colonies.
- Blood agar → hemolytic colonies of *Y.pseudotuberculosis*.
- Media containing bile salts are selective media.

BIOCHEMICAL ACTIVITY

- Glucose, maltose, mannitol, sucrose or rhamnose are fermented with the production acid but no gas.
- *Y.enterocolitica* ferments sucrose, while *Y.pseudotuberculosis*doesn't.

- *Y.pseudotuberculosis* ferments rhamnose, while *Y.enterocolitica* doesn't.
- Urease and H_2S production is characteristic.
- Lactose is not fermented.

ANTIGENIC STRUCTURE

Y.enterocolitica includes 34 serogroups (the most of human infections are caused by O3, O9, and O8). *Y.pseudotuberculosis* includes 6 serogroups (the most of most human infections are caused by O1).

ECOLOGY AND RESISTANCE

- Yersinia is resistant bacteria in nature (it remains viable for several months in cold, moist environment, soil).
- Yersinia is obligate pathogen and facultative intracellular parasite of monocytes/macrophages system.

FACTORS OF PATHOGENICITY

1. Fimbriae (for adhesion).
2. Endotoxin (LPS).
3. Extracellular enzymes (for invasion).
4. Enterotoxins: ST, LT.

Yersinia can produce invasive proteins (**Yop proteins**) and structures for **type III bacterial secretion system** (injectisome, or "needle" complex) which provides microbial adherence to host cells and subsequent invasion by endocytosis (→ survival of bacteria and their dissemination).

CAUSED DISEASES

❖ **Intestinal yersiniosis** (caused by *Y.enterocolitica).*

❖ **Pseudotuberculosis**(caused by *Y.pseudotuberculosis*).

Intestinal yersiniosis can be appeared in three characteristic clinical forms. The first type occurs in small children as self-limited *gastroenteritis* or *enterocolitis* (febrile diarrhea with pus and blood). The second is *mesenteric lymphadenitis* and inflammatory terminal *ileitis* in older children that can mimic appendicitis. The third type is a systemic disease typically in adults, often characterised by bacteremia, meningitis, reactive arthritis and erythema.

Symptoms of pseudotuberculosis are similar to intestinal yersiniosis (fever and right-sided abdominal pain from mesenteric lymphadenitis), except that the diarrhea is often absent. Prolonged asymptomatic bacteremia can also occur and be responsible for fatal reaction from blood transfusion (yersinia can multiply in blood stored in cold).

EPIDEMIOLOGY

- Source of infection is animals (wild and domestic animals, rodents); for intrahospital yersiniosis — human (donated blood).
- Mechanisms of transmission are fecalo-oral (unpasteurized milk, pork) and contact with animals for zoonotic form of disease; blood transfusion — for intrahospital form of yersiniosis.

- Immunity is type-specific.
- No vaccination.
- Prevention: proper sanitation, examination of donor blood before use.
- Antibiotic therapy is common against Gram-negative rods.

Klebsiella

- **Gram-negative** nonmotile lactose fermentating **rods** which produce polysaccharide capsule*in vivo* and *in vitro* (in media).
- *Klebsiella pneumoniae* can be divided into 70 serogroups by K-Ag.
- Methophilic, facultative anaerobes.
- Large mucoid S-colonies on blood agar; on DDM → lactose-positive colonies.
- Pathogenic factors: endotoxin (LPS), fimbriae, polysaccharide capsule, enterotoxins (LT, ST).
- *K.pneumoniae*is found as commensal of GIT and upper respiratory tract. Diseases caused by *K.pneumoniae* (common name — **klebsiellosis**) are: 1) Pneumonia (community-acquired; most common in patients with chronic lung disease, diabetes, alcoholism; sputum is usually bloody like "currant jelly"). 2) Other PSI, often nosocomial: UTI (catheter-related), septicemia, meningitis, surgical wound infections. 3) Gastroenteritis (food toxico-infection).
- *K.rhinoscleromatis* causes **rhinoscleroma** of nose and pharynx (chronic granulomatous hypertrophy and deformity of nose leading to the airways obstruction).
- *K.ozaenae* causes **ozena** (atrophic rhinitis characterized by foul smelling nasal discharge).

Proteus

- **Gram-negative** nonlactose-fermentating **rods** which arehighly motile with peritrichous flagella ("swarming" growth on the surface of agar during prolonged cultivation).
- Methophilic, facultative anaerobes.
- S-colonies are seen in young cultures (DDM, blood agar). Proteus is characterized by putrefactive odor ("fishy" or "seminal").
- *P.mirabilis* is indole negative, *P.vulgaris* is indole positive.
- Pathogenic factors: endotoxin, fimbriae, enterotoxins.
- Powerful urease raises urine pH that provokes kidney stones.
- Normal intestinal commensals and widely distributed in nature.
- Proteus is opportunistic pathogen responsible for UTI and other pyoseptic infections, often nosocomial (*P.mirabilis*). *P.vulgaris* is found much less in human infections.

Lecture 13. AEROBIC AND FACULTATIVELY ANAEROBIC GRAM-POSITIVE RODS AND ACTINOMYCETES

MYCOBACTERIA

TAXONOMY

Domain: Bacteria
Phylum: Actinobacteria
Order: Actinomycetales
Family: Mycobacteriaceae
Genus: **Mycobacterium**

Species: *Mycobacterium tuberculosis*(MBT), *M.bovis, M.leprae,* etc. **Non-tuberculous mycobacteria(NTM)** are*M.kansasii, M.microti, M.xenopi, M.avium-intracellulare* (MAC), etc.

Classification of mycobacteria

► Duration of growth: slowly- and rapidly-growing species. Mycobacteria that form colonies clearly visible to the naked eye within seven days on subculture are termed **rapid growers**, while those requiring longer periods are termed **slow growers**. Ex.: MBT is slow grower, NTM are rapid growers.

► Pigmentation: **photochromogens** produce nonpigmented colonies when grown in the dark and pigmented colonies only after exposure to light and reincubation (e.x., *M.kansasii*);**scotochromogens** produce deep yellow to orange colonies when grown in the presence of either the light or the dark (e.x.,*M.xenopi*); **non-chromogens** produce nonpigmented in the light and dark (e.x., MBT, *M.bovis*).

► Pathogenicity: obligate pathogens and opportunistic pathogens.

Nontuberculous mycobacteria (NTM) are saprophytes, opportunistic mycobacteria, causativeagents of mycobacterioses. Clinical forms of mycobacterioses are pulmonary disease resembling tuberculosis, lymphadenitis, skin disease, generalized infection. NTM are also known as environmental mycobacteria, atypical mycobacteria and mycobacteria other than tuberculosis (MOTT).

Obligate pathogenic mycobacteria are causative agents of:

- Tuberculosis (TB): *M.tuberculosis* (the most of cases of TB), *M.bovis*(cattle TB) and *M.africanum* (rare). They are called *MBT-complex*.
- Leprosy: *M.leprae* and *M.lepramurium*.

MORPHOLOGY

- **Gram-positive**, **acid-fast bacilli(AFB)**.
- They are nonmotile, nonsporing, noncapsulated slender **rods**.
- Can stick together due to cord-factor.
- Special staining is Zielh-Neelsen, Kinyoun, fluorescent stainings.
- Characteristic feature of mycobacteria is high amount (60 %) of lipids and glycolipids in the cell wall (lipoarabinogalactan, mycolic acids, cord factor, glycolipids/mycosides, wax D, etc.); also tuberculin (tuberculoprotein) is present.
- Mycobacteria can form L-forms.

BIOLOGICAL PROPERTIES

- Obligate aerobes, capnophiles.
- Mesophiles.

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

- Extremely long reproductive cycles (growth of *M.lepra*emay take more than 20 days to proceed through one division cycle; for comparison, *E.coli* strains take only 20 minutes) are making bacteriological method a slow process → cultivation of MBT is taking of 3–8 and more weeks.
- R-colonies are virulent. They are dry, irregular, with wrinkled surface, creamy white or yellowish, resemble cauliflower like or warts.
- Media with high lipid content: Lowenstein-Jensen, Middlebrook semi-synthetic, Finn,Soton's synthetic media.
- Pryce's microculture method on narrow glass slides is available for rapid cultivation of mycobacteria in the citrate blood. Virulent strains of mycobacteria often grow as twisted rod line accumulations which are called "serpentine cords".

BIOCHEMICAL ACTIVITY

- Niacin test (positive with MBT and negative with *M.bovis*).
- Neutral red test (positive with virulent strains of MBT).
- Catalase test (tubercule bacilli are peroxidase positive and weakly catalase positive).

ANTIGENIC STRUCTURE

Lipids, contained in mycobacteria, are regarded as weak antigens (haptens). But in complex with mycobacterial proteins they can induce CMI. Also mycobacterial antigens activate production of specific antibodies usually in low titers.

Tuberculin is a specific antigenic complex composed of various tuberculoproteins. It causes hypersensitivity reactions (DTH) evaluated by tuberculin skin test.

ECOLOGY AND RESISTANCE

- *M.tuberculosis* is obligate pathogen and facultative intracellular parasite of macrophages.
- Mycobacteria show high resistance in the environment.
- High concentration of lipids in the cell wall has been associated with marked resistance of MBT to many factors:
  - Impermeability to stains and dyes.
  - Resistance to chemotherapeutic preparations and disinfectants.
  - Resistance to killing by acidic and alkaline compounds.
  - Resistance to osmotic lysis via complement.
  - Resistance to phagocytosis.
  - Resistance to drying (survive for long periods in dried sputum).
- Mycobacteria are sensitive to sunlight and UV-irradiation.

FACTORS OF PATHOGENICITY

Classical pathogenic factors are absent (capsule, toxins, enzymes).

1. The cell wall lipids and tuberculoproteins are playing the main role in pathogenesis of TB. They responsible for:

- Resistance to the humoral immune factors (complement).
- Intracellular persistence of MBT in nonactivated macrophages (inhibition of phagocytosis by means of prevention of phagosome-lysosome fusion).
- Induction of DTH ("tuberculin allergy") and chronic immune inflammatory cell-mediated response with **granuloma** formation.
- Mycolic acids render toxic effect against host tissues.

2. Slow generation time of MBT (immune system may not readily recognize the bacteria or be triggered sufficiently to eliminate them).

3. Cord factor is primarily associated with virulent strains of MTB. It is responsible for toxic effect by inhibition of biological oxidation in the host cells.

CAUSED DISEASES

Mycobacterial diseases are: 1) **Tuberculosis** (TB); 2) **Leprosy**; 3) **Mycobacterioses**; 4) Skin infections (*M.ulcerans* produces **Buruli ulcer** – chronic ulcers on the skin which heal with disfigurating scars).

Clinical forms of TB:

- Primary and secondary TB.
- Pulmonary and extrapulmonary TB.

Characteristics and pathogenesis of primary tuberculosis:

- In the majority of cases MBT enters the lung in droplets, where it is phagocytosed by alveolar macrophages. MBT multiplies virtually unrestricted within inactivated alveolar macrophages due to their ability to inhibit formation of phagolysosomes.
- Within 10–14 days a reactive inflammatory complex develops, the so-called "primary focus" (PF) from which the MBT move to the regional lymph nodes, where they reproduce and stimulate CMI, which in turn results in clonal expansion of specific T-lymphocytes and attendant lymph nodes swelling. **Ghon's complex** or primary tuberculous complex (PF + lymphadenitis) develops between 6 and 14 weeks after infection.
- At the same time **granulomas (tubercles)** areforming at the primary infection site and in the affected lymph nodes. The center of the tubercle is characterized by **"caseation necrosis"** meaning its semi-solid consistency (to the naked eye, this has the texture of soft, white cheese). Granuloma prevents dissemination of the mycobacteria and provides a local environment for interaction of immune cells.
- T-cell activation and release of cytokines (ɣ-IFN) leads to activation of macrophages which now are capable of destroying MBT.
- At this stage the individual becomes tuberculin-positive. This positive tuberculin reaction is the result of DTH development.
- AMI will not aid in the control of MBT infection because it is intracellular and resistant to humoral factors.

• Mycobacteria may invade bloodstream and disseminate to almost any anatomical location but usually involve the genitourinary system, bones, joints, lymph nodes and peritoneum.

• The further course of disease depends on outcome of the “battle” between MBT and CMI. The host eventually prevails in over 90 % of cases: the granulomas and foci fibrose, scar and calcify and the infection remains clinically silent (**LTBI**or**latent TB-infection**).

Characteristics and pathogenesis of secondary TB:

• In about 10 % of infected persons the TB reactivates within several months or years after primary infection (**endogenous reactivation**). **Exogenous reinfection**with another strain of MBT is also possible.

• Reactivation begins from *caseation necrosis* in the center of granuloma that may progress to cavitation (formation of caverns).

• General signs and symptoms of TB include fever, chills, night sweats, loss of appetite, weight loss and fatigue. Symptoms may include chest pain and prolonged cough producing sputum, usually bloody. About 25 % of people may not have any symptoms ("asymptomatic"). In some cases, the infection spreads outside the respiratory organs, causing other kinds of TB. Extrapulmonary TB occurs more commonly in immunosuppressed persons and young children. Extrapulmonary infection sites include the pleura, CNS (tuberculous meningitis), lymphatic system (neck), genitourinary system (kidney tuberculosis), and the bones (osseous tuberculosis or osteomyelitis) and joints. A potentially more serious, widespread form of TB is called "disseminated" TB, commonly known as miliary tuberculosis.

EPIDEMIOLOGY

• Source of infection is humans (cattle for *M.bovis*).

• Mechanisms of transmission are airborne (*M.tuberculosis*) and fecal-oral (infected milk with *M.bovis*).

Tuberculosis is the second most common cause of death from infectious disease (after those due to HIV/AIDS). Tuberculosis is more common in developing countries.

IMMUNITY, TREATMENT AND PREVENTION

• Nonsterile immunity lasts as long as TB remains in the organism.

• Basis of immunity is tuberculin allergy (DTH).

• Vaccine BCG (bacilli of Calmette and Guerin) consists of attenuated alive bovine mycobacteria (*M.bovis*). Vaccination schedule: on 3–4 day of life, than before school.

• The most commonly used antituberculous drugs areisoniazid, rifampicin, etc. Treatment can be prolonged, taking several months.

• **Medication resistance problem**. Primary resistance occurs when a person becomes infected with a resistant strain of TB. Secondary resistance results from inadequate treatment. Drug-resistant TB is a serious public health issue in

many countries, as its treatment is longer and requires more expensive drugs. Multi-drug resistant TB (MDR-TB) is defined as resistance to the two most effective first-line TB drugs: rifampicin and isoniazid. Extensively drug-resistant TB (EDR-TB) is also resistant to three or more of the six classes of second-line drugs. Totally drug-resistant TB, which was first observed in 2003 in Italy, but not widely reported until 2012, is resistant to all currently used drugs.

Mycobacterium leprae

- *M.leprae* is obligate human pathogen.
- *M.leprae* is **Gram positive**, slightly curved or straight **rods**, arranged singly, in parallel bundles like rolls of cigarettes in a packet or in globular masses. They are **acid-fast** but less than the tubercle bacilli. Microorganisms are nonsporing, noncapsulated and nonmotile.
- *M.leprae* has not yet been grown on artificial media or tissue culture. Armadillos can be used for cultivation.
- **Leprosy** is a slow, chronic granulomatous disease. Pathogenesis of leprosy is identical to TB (formation of leprous granulomas). Incubation period is long (3 to 15 years). It is anthroponosis. Portal of entry is most likely through damaged skin, cuts and nasal mucosa. Prolonged close contact with infected patients is necessary for transmission of the disease. The bacilli infiltrate the skin and cutaneous nerves forming visible lesions.
- There are 4 types of leprosy: (1) Lepromatous form (malignant, progressive course with nodular skin lesions and cord like nerve thickenings that finally lead to neuroparalysis); (2) Tuberculoid form (benign, nonprogressive form characterized by spotty dermal lesions); (3) Dimorphous form (lesions possess characteristics of both previous types); (4) Intermediate form (unstable tissue reaction which is not characteristic for certain type of leprosy).
- Infection induces CMI and AMI, but circulating antibodies are without any effect. CMI plays the major part in determining the response of a host to the infection.
- Prevention is nonspecific. Dapson and rifampicin are drugs of choice.

ACTINOMYCETES

TAXONOMY

Domain: Bacteria
Phylum: Actinobacteria
Class: Actinobacteria
Order: Actinomycetales

Family: Actinomycetaceae	Family: Nocardiaceae
Genus: **Actinomyces**	Genus: **Nocardia**
Species: *Actinomyces israelii, A.bovis, A.naeslundii*, etc.	Species: *Nocardia asteroides, N.brasiliensis*, etc.

MORPHOLOGY

- **Gram-positive rods** which tend to grow in the form of **branched filaments**. Aggregation of filaments forms **mycelium**.

• Yellowish sulfur granules or **druses** (1-2 mm) can be observed macroscopically in actinomycetes pus (they are bacterial macrocolonies which consist of a dense network of thin filaments, surrounded by a peripheral, swollen radiating club shaped structures).

BIOLOGICAL PROPERTIES

• Facultative or obligate anaerobes.
• Mesophiles.

MEDIA FOR CULTIVATION AND CULTURAL PROPERTIES

Thioglycollate liquid medium, brain-heart infusion agar.

A.israelii produces mycelial microcolonies only during the first days ("spidery colonies"). Whitish macrocolonies, often with a rough surface, begin to appear after two weeks. Actinomyces colonies form fungus-like branched networks of hyphae. The aspect of these colonies initially led to the incorrect assumption that the organism was a fungus and to the name "*Actinomyces*" or ray fungus.

In liquid media → "fluffy balls" at the bottom of tube.

ECOLOGY AND RESISTANCE

• The most of actinomycetes are saprophytes that live in soil.
• Facultative pathogens (normal flora of mouth, intestine and vagina).

FACTORS OF PATHOGENICITY

• Highly adhesive and invasive bacteria. Most important pathogenic factors are cell wall components (teichoic acids, peptidoglycan, etc.).
• Invasion of tissues with compromised oxygen supply.
• Induction of DTH and granuloma formation.

CAUSED DISEASES

Actinomycosis is endogenous infection. It is chronic granulomatous disease characterized by the indurated swellings, mainly in connective tissue, suppuration and discharge of "sulfur granules". Actinomycosis is usually mixed infection with bacteria which may enhance the pathogenic effect of actinomycetes (bacteroides, staphylococci, anaerobic streptococci, etc.).

The main clinical forms of actinomycosis are:

• Cervicofacial ("lumpy jaw") actinomycosis with indurated lesions on the cheek and submaxillary regions.
• The symptoms of thoracic actinomycosis resemble those of a sub-acute pulmonary infection: mild fever, cough and purulent sputum (it results from aspiration of saliva).
• Abdominal actinomycosis (lesions are usually around cecum).
• Genital (pelvic) actinomycosis is a rare occurrence in women that results from colonization of an intrauterine device.
• Some Actinomyces species are involved in the development of *caries, gingivitis, periodontitis*and *mycetoma*.

EPIDEMIOLOGY

• Source of infection is frequently endogenous (normal flora) or soil (exogenous). Young men are most commonly affected.

• Mechanisms of transmission are contact and autoinfection. Trauma, foreign bodies or poor hygiene may favor tissue invasion. Actinomycosis occurs throughout the world but its incidence is more common in rural areas and in agricultural workers.

IMMUNITY, TREATMENT AND PREVENTION

• Nonlong-lasting immunity.

• Treatment includes both surgical and antibiotic measures. The antibiotic of choice is aminopenicillin.

Nocardia

• Nocardia resemble actinomycetes morphologically but they are aerobic in respiration.

• All species are **Gram-positive** and some such as *N.asteroides* and *N.brasiliensis* also **acid fast**.

• Nocardia is frequently found in soil and infection may be exogenous (inhalation of soil/dust particles or traumatic implantation).

• Clinical forms of **nocardiosis** are cutaneous, subcutaneous and systemic lesions in human. Cutaneous infection may lead to local abscesses, cellulitis or lymphocutaneous lesions. Subcutaneous nocardiosis is mycetoma (draining abscesses). Systemic infection usually manifests primarily as pneumonia, lung abscess or other lesions resembling TB. Metastatic manifestations may involve the brain, kidneys and other organs. Systemic nocardiosis occurs more often in immunodeficient persons.

• Common chemotherapeutic preparations are sulfonamides or trimethoprim/sulfamethoxazole.

CORYNEBACTERIA

TAXONOMY

Domain: Bacteria
Phylum: Actinobacteria
Class: Actinobacteria
Order: Actinomycetales
Family: Corynebacteriaceae
Genus: **Corynebacterium**
Species: *Corynebacterium diphtheriae, C.xerosis, C.ulcerans*, etc.

Diphtheroids (*C.pseudodiphthericum, C.xerosis*) are opportunistic corynebacteria, which are saprophytes or normal flora of animals and humans. They can cause PSI (conjunctivitis, endocarditis, etc.) or diphtheria-like infections (*C.pseudodiphthericum, C.ulcerans*).

MORPHOLOGY

• **Gram-positive**, club-shaped, nonmotile, nonsporing, noncapsulated **rods**, arranged in pairs, palisades and V or L clusters. This has been called *Chinese letters* arrangement.

• The often contain **volutin granules** (metachromatic nutritional granules) at the both ends of rods. Special stainings for revealing of granules are Loeffler methylene blue, Neisser and Albert.

BIOLOGICAL PROPERTIES

• Obligate aerobes.
• Mesophiles.
• Auxotrophs.

MEDIA FOR CULTIVATION AND CULTURAL PROPERTIES

• Loeffler coagulated serum agar (for revealing of volutin granules).
• Tinsdale medium (cystine-tellurite serum agar).
• Tellurite whole blood agar.
• There several biotypes of *C.diphtheriae – mitis*, *gravis* and *intermedius*. The names were originally proposed to relate to the clinical severity of disease; however this association is not constant.

Table 20 — Cultural and biochemical differentiation of Corynebacteria

Features	***C.diphtheriae***		**Diphtheroids**
	Gravis biotype	**Mitis** biotype	
1. Colonies	R-colonies (flat colonies with raised dark center and radial striation like "daisy head")	S-colonies (flat colonies with central elevation like "poached egg")	S-colonies
2. Hemolysis	-	+	-
3. Starch decomposition	+	-	-
4. Cystinase test	+	+	-
5. Urease test	-	-	+
6. Toxigenicity	95–99 % of strains	80–85 % of stains	Nontoxigenic

Biotype gravis is associated with high fatality rate, while mitis infections are less lethal. Mitis is predominant in endemic areas; while gravis tends to be epidemic (other differences are seen in table 20).

BIOCHEMICAL ACTIVITY

Diphtheria bacilli ferment carbohydrates with the production of acid but no gas. Virulent strains of *C.diphtheriae* have been found to ferment sucrose. Biotype gravis ferments starch, while mitis cannot.

ANTIGENIC STRUCTURE

Gravis stains have been classified into 13 types and mitis into 40 types.

ECOLOGY AND RESISTANCE

• *C.diphtheriae* is obligate pathogen and extracellular parasite.
• Corynebacteria are relatively resistant in nature.

FACTORS OF PATHOGENICITY

• Factors of adhesion and colonization (fimbriae) → epithelium of nasopharynx, oropharynx, skin.

• Corynebacteria are not invasive.

• Cord-factor (inhibits of cellular respiration).

• **Diphtheria exotoxin**is cytotoxin which inhibits protein synthesis by adding ADP-ribose to EF-2.

Mechanism of exotoxin action:

(a) Local effect (in nasopharynx/oropharynx) → formation of dirty grey **pseudomembrane** (made up of necrotic cells, bacteria, leukocytes and fibrinous exudate); possible extension of pseudomembrane into larynx/trachea and airway obstruction → fatal asphyxia.

(b) Systemic effect (circulation in blood or toxenemia) → severe intoxication, affection of heart (myocarditis), neurons (demyelinating polyneuropathy with involvement of cranial and peripheral nerves).

▪ Only virulent strains of *C.diphtheriae* can produce exotoxin and responsible for development of diphtheria. Avirulent (nontoxigenic) strains are common for convalescents, contacts and carries.

▪ Toxin-producing strains have β-prophage carrying genes for the exotoxin (β-corynephage). Nontoxigenic strains may be rendered toxigenic by infecting them with this phage due to **lysogenic conversion**. The toxigenicity remains only as long as the bacillus is lysogenic.

C.ulcerans and *C.pseudotuberculosis* can carry *tox*-gene that encodes diphtheria exotoxin; thereby they can also cause disease in rare cases.

CAUSED DISEASES

Diphtheria is infection of URT which leads to fever, sore throat, malaise, pallor, sleepiness due to intoxication.

Clinical forms of diphtheria depend on localization of the infectious process: nasal, ***nasopharyngeal***, ***tonsillar***, laryngeal, genital-vulval, vaginal and cutaneous. According to the clinical severity diphtheria may be classified as: hypertoxic (severe toxenemia with marked adenitis — "bull neck diphtheria"), septic (ulceration, cellulitis), hemorrhagic.

• Diphtheria is a *toxenemia.* Bacteria remain confined to the site of entry, where they multiply and produceexotoxin.

• The toxin causes local inflammation and necrosis → formation of grey-yellowpseudomembrane→ possible extension downwards to the larynx where it can block the passage of air and cause death from asphyxia (mechanical obstruction or *croup syndrome*).

• The common complications of diphtheria are: asphyxia, myocarditis, palatine and ciliary paralysis, otitis, pneumonia.

EPIDEMIOLOGY

• Source of infection: sick persons and carriers (mostly, children).

• Mechanisms of transmission are aerogenic, contact, food-borne (rare,for *C.ulcerans*with infected milk).

- Immunity is long-lasting (antitoxic).
- Vaccination by DPT (DT) (diphtherial component is anatoxin or toxoid). Passive immunization can be given for contacts (subcutaneous administration of antidiphtheric serum — ADS; it is horse serum, precaution against anaphylactic shock should be observed).
- Specific treatment of diphtheria consists of antitoxic and antibiotic therapy. ADS should be given immediately when a case is suspected as diphtheria, as the fatality rate increases with delay o in starting antitoxic treatment. Antibiotics of choice are β-lactams and macrolides.

Listeria monocytogenes

- **Gram-positive** motile **rods**.
- Aerobic, can reproduce at low temperature ("cold enrichment").
- Media for cultivation is selective blood agar.
- Widespread distribution: animals, unpasteurized milk products, soil, plant.
- Opportunistic pathogen and facultative intracellular parasite.
- Factors of pathogenicity: factors of adhesion (fimbriae), listeriolysin O (hemolysin, which inhibits phagocytosis), protein internalin (for invasion). Listeria multiplies in the cytoplasm of the infected cell and disseminate intracellularly between cells. Polymerization of the actin of the infected cell forms "actin tail" that move listeria towards the membrane. Then "listeriopods" are forming (long membrane protuberances containing listeria). Neighboring cells engulf the listeriopods and the process is repeated.
- Clinical forms of **listeriosis**:

1) In immunocompetent persons → asymptomatic infection or clinically apparent infection (*gastroenteritis* or mild flu).

2) In immunocompromised persons → *sepsis*,*meningitis, meningoencephalitis* (in renal transplant patients, humans with cancer or T-cell defects, in alcoholics, during cortisone therapy, pregnancy, in elderly persons and infants).

3) *Neonatal listeriosis*or *granulomatosis infantiseptica*(spontaneous abortion, sepsis with high mortality, disseminated granulomas with subsequent necrosis).

- Source of infection is zoosapronosis. Mechanisms of transmission are alimentary (milk, soft cheese, deli meats, cabbages), contact, aerogenic, transmissive (ticks for cattle), transplacental.
- Prevention is a proper storage of food, keeping of hygienic conditions.
- Listeriosis is a rare disease characterized by sporadic occurrence but occasional gastrointestinal epidemics may occur.

Lecture 14. CAUSATIVE AGENTS OF HIGH-RISK INFECTIONS OF THE BACTERIAL ETIOLOGY. OBLIGATELY ANAEROBIC BACTERIA

VIBRIO

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Vibrionales
Family: Vibrionaceae
Genus: **Vibrio**
Species: *Vibrio cholera, V.parahaemolyticus*

MORPHOLOGY

Gram-negative nonsporing **comma-shaped rods**.They are actively motile with a single polar flagellum. In stained films of mucous flakes the vibrios are seen in parallel rows ("fish in stream" arrangement).

BIOLOGICAL PROPERTIRES

- Obligate aerobes.
- Mesophiles.
- Alkaliphiles (readily grow at pH 8,0–9,5).
- The growth of vibrio species is maintained by increased concentrations of NaCl (0.5–2 %).

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

- S-colonies are virulent (moist, transparent disks with a bluish tinge in transmitted light).
- Vibrio can grow on simple media (MPA and MPB).
- DDM → lactose-negative colonies.
- Alkaline peptone water → surface pellicle.
- Alkaline **b**ile **s**alt agar (BSA) → small, translucent S-colonies.
- TCBS medium (**t**hiosulfate, **c**itrate, bile salts, **s**ucrose agar) → large yellow S-colonies.

BIOCHEMICAL ACTIVITY

- Cholera vibrios ferment carbohydrates producing acid but no gas.
- Lactose-negative bacteria.
- *Heiberg* biochemical classification → 6 groups by fermentation of sucrose, arabinose, mannose; *V.cholerae* belongs to Group I,which can ferment sucrose and mannose but not arabinose.
- "Cholera red reaction" (formation of nitroso-indole revealed by adding of sulfuric acid to peptone water culture of *V.cholerae*).
- Catalase and oxidase are positive.

ANTIGENIC STRUCTURE

- H-Ag.
- O-Ag.

Antigenic classification of choleric vibrios is based on O-Ag structure and includes more ≥ 200 serogroups. Causative agents of epidemic cholera belong to O1 and O139 serogroups. Serotypes of O1-serogroup may be agglutinated by O1-antiserum. This group consists of two biotypes: **classical** and **El Tor**. Each biotype includes 3 serotypes: Ogawa, Inaba and Hikojima. For example, *V.cholerae* serogroup O1, biotype El Tor, serotype Ogawa.

V.cholerae O139-serogroup ("Bengal" serotype) was discovered in 1992.

Other serogroups contain remaining strains and referred to as nonO1-serogroups (cannot be agglutinated by O1-antiserum). They are called **n**on**ag**glutinable vibrios (NAG vibrios); also they considered as nonpathogenic and hence also called **n**on**c**holeric **v**ibrios (NCV).

ECOLOGY AND RESISTANCE

- *V.cholerae* is obligate pathogen and extracellular parasite.
- Vibrios resist alkalinity, but not heating, drying and acidity. Its survival in water is influenced by pH, temperature, presence of organic pollutions, bacteriophages and other factors. In general El Tor vibrios survive longer than classical cholera vibrio biotype.
- Gastric juice of normal acidity kills vibrio in a few minutes (in achlorhydric gastric juice they may survive for 24 hours).
- Cholera vibrios can survive in feces for about a month; also they readily survive at low temperature.
- They are sensitive to disinfectants and antibiotics.

FACTORS OF PATHOGENICITY

1. Cholera exotoxin (**cholerogen**) → accumulation of cAMP → extraction of water and electrolytes from tissue and blood into the lumen of the intestine → watery diarrhea. Cholera exotoxin is encoding by specific bacteriophage (CTXφ). Production of cholerogen is carried out only by toxigenic strains of *V.cholerae* due to lysogenic conversion.
2. Enterotoxins (ST/LT).
3. Endotoxin (LPS).
4. Invasion enzymes (hyaluronidase, collagenase, fibrinolysin, neuraminidase, etc.).
5. Fimbriae (toxin-coregulated pili responsible for intestinal adhesion and colonization).
6. Hemagglutinins (surface proteins that agglutinate RBCs).

CAUSED DISEASES

Cholera (asiatic cholera or epidemic cholera) is characterized by severe dehydration, intoxication and gastroenteritis.

The primary symptoms of cholera are profuse, painless diarrhea and vomiting of clear fluid. These symptoms usually start suddenly, one to five days after ingestion of the bacteria. The diarrhea is frequently described as "rice water" and may have a fishy odor. An untreated person with cholera may produce 10–20 liters of diarrhea a day with fatal results. Cholera has been nicknamed the "blue death" due to a patient's skin turning a bluish-gray hue from extreme loss of fluids. The typical symptoms of dehydration include low blood pressure, poor skin turgor, wrinkled hands, sunken eyes and a rapid pulse. There are many asymptomatic or atypical infections during outbreaks and epidemics.

EPIDEMIOLOGY

- Source of infection is humans or aquatic environment (*V.cholerae* is found in shellfish and plankton).
- Mechanism of transmission is fecalo-oral (especially hand contamination of stored drinking water). Cholera is rarely spread directly from person to person.

Cholera is endemic for India. The first spread of choler to Europe and USA began in 1817, such that by the early 20^{th} century, six waves (pandemics) occur in the world.In 1961, the El Tor biotype produced a major epidemic in the Philippines to initiate seventh global pandemic.

In 1992, large 8 epidemics in Bangladesh caused by *V.cholerae* O139 “Bengal” (11 countries of southern Asia had been involved).

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is relatively nonlong-lasting.
- Vaccines: inactivated, anatoxin, live attenuated.
- Prevention: proper sanitation, protection of water supply sources and prevention of water and foodstuff pollution.
- The treatment of cholera consists essentially of the prompt and adequate replacement of lost fluid and electrolytes (oral administration or intravenous in severe cases). Antibacterial therapy is of secondary importance (tetracyclines).

Vibrio parahaemolyticus

- It is enteropathogenic **halophilic vibrios** (high requirement of NaCl)
- Natural habitat in sea water and marine life (fishes, shrimps, crabs, mollusks, etc.).
- It grows only in media containing NaCl (2–8 %) such as high salt blood agar. On TCBS agar the colonies are green.
- Kanagawa phenomenon: strains isolated from environmental sources are nonhemolytic while strains from patients are hemolytic.
- No enterotoxins have been identified. The vibrio is believed to cause *enteritis* by invasion of the intestinal epithelium.
- *V.parahaemolyticus* is an important cause of food poisoning throughout the world. Abdominal pain, bloody diarrhea, vomiting and fever are common signs. Dehydration is of moderate degree and recovery occurs in 1–3 days.

BRUCELLA

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Alphaproteobacteria
Order: Rhizobiales
Family: Brucellaceae
Genus: **Brucella**
Species: *Brucella melitensis*, *B.abortus*, *B.suis*, etc.

MORPHOLOGY

Gram-negative nonsporing **coccobacteria** or **rods**, nonmotile, some strains produce soft polypeptide capsule.

BIOLOGICAL PROPERTIRES

- Obligate aerobes.
- Capnophilic (*B.abortus*).
- Mesophiles.

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

The growth of brucella is extremely slow (they can grow until 8–15 days and more) and the culture has a risk to laboratory personnel.

- S-colonies are virulent (small, moist, transparent, glistering), but later S-colonies mutate to a rough type which is associated with changes in antigenic structure.
- On the blood agar colonies are nonhemolytic.
- Species media for cultivation: Castaneda medium, tryptose soy agar, liver infusion media, serum potato infusion agar.
- All brucella can be cultivated in the yolk sac of chicken embryos.

BIOCHEMICAL ACTIVITY

- No carbohydrates are fermented.
- Urease production is characteristic.
- H_2S production (*B.abortus* and *B.suis*).
- Different sensitivity of species to dyes (basic fuchsin and thionin).

ANTIGENIC STRUCTURE

O-Ag contains two antigenic determinants (A and M):

- *B.abortus* possesses predominant A-Ag.
- *B.melitensis* possesses predominant M-Ag.
- *B.suis* possesses both Ag determinants.

ECOLOGY AND RESISTANCE

- Obligate pathogen and facultative intracellular parasite (*B.melitensis* is the most virulent among all brucella species).
- Brucella is highly resistant in nature (may survive in soil, meat for several weeks). Their resistance for drying renders brucella stable in aerosol form, facilitating aerogenic transmission.

• They are sensitive to all conventional disinfectants.

FACTORS OF PATHOGENICITY

1. Capsule (for some strains of *B.melitensis* only).

2. Fimbriae (for adhesion).

3. Endotoxin (LPS) → plays a key role in pyrogenicity and resistance to phagocytosis.

4. Extracellular enzymes (for invasion).

5. Facultative intracellular parasites (localized and persist macrophages of spleen, liver, lymph nodes and bone marrow; can also target the reproductive tract) → long persistence → chronic septicemia or bacteremia.

6. Induction of DTH (formation of granulomas and necrosis of tissues).

►*B.melitensis* is the most aggressive species which produces malignant type of human disease. *B.suis* is of intermediate pathogenicity and *B.abortus* produces less serious disease in humans.

CAUSED DISEASES

Brucellosis (**Bang's disease**)can affect any organ/organ system, and 90 % of patients have a cyclical fever ("*undulant fever*"). The duration of the disease can vary from a few weeks to many months or even years.

If untreated, the disease becomes chronic (with minimal pyrexia).

Other symptoms include chills, headache, fatigue, arthralgia/myalgia, depression, weight loss (anorexia), nervous irritability, exhaustion, constipation and liver dysfunction (hepatomegaly). Foul smelling perspiration is considered a classical sign (drenching nocturnal sweats).

Later complications may include arthritis or epididymoorchitis, spondylitis, neurobrucellosis (depression and mental fatigue), liver abscess formation, and endocarditis, the latter potentially fatal.

Clinical forms of brucellosis:

1. Acute form; mostly is due to *B.melitensis*.

2. Chronic form (disease more than 1 year): usually in older people. Chronic brucellosis is hard to define; length, type and response to treatment are variable.

3. Latent form (with serological but no clinical evidence).

EPIDEMIOLOGY

• Source of infection is highly contagious zoonosis (domestic livestock). *B.abortus* is isolated from cattle, *B.suis*— from pigs and *B.melitensis* — from sheep and goats. Pathogenic brucella species can cause abortion in female animals by colonization of placenta and sterility in male animals.

• Mechanisms of transmission are contact with secretions of animals; alimentary (raw milk, soft cheese, undercooked meat); inhalation of aerosols with animal wool or in laboratory, contact with animals (especially with animal vaginal discharges, fetuses, placenta, and urine); accidental skin penetration or abrasion (farmers, slaughterhouse workers, veterinarians).

Brucellosis is also considered an occupational disease because of a higher incidence in people working with animals.

Brucellosis is a worldwide in distribution and is endemic in certain areas such as Mediterranean countries.

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is relative; reinfections/relapses are possible.
- Vaccines have been developed for use in animals. They can be administered to protect the contact persons as well as the personnel with occupational risk of brucellosis.
- Prevention – checking of the dairy animals and pasteurization of milk.
- Antibiotics of choice are tetracyclines or β-lactams.
- Chronic cases can be treated by killed vaccine that activates immunity.

Yersinia pestis

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: **Yersinia**
Species: *Y.pestis*

MORPHOLOGY

Gram-negative nonsporing **coccobacteria** with bipolar staining arranged singly, in short chains or in small groups. *Y.pestis* is nonmotile and produce polypeptide capsule. Pleomorphism is very common.

BIOLOGICAL PROPERTIRES

- Facultative anaerobes.
- Psychrophiles (optimal temperature is 28–29 °C). 37°C is optimal for capsule formation.

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

- They grow in simple media. \
- In the solid media after 8–12 hours of incubation S-colonies are visible (less virulent, small, delicate, translucent, resembles piece of crushed glass). After 18 hours, colonies enlarged in size and become R-colonies(more virulent and characterized by lace-pocket shape).
- In peptone broth *Y.pestis* shows flocculent growth at the bottom and along the sides of the tube (delicate pellicle may form layer).
- When bacteria are grown in a broth with a little amount of oil on the top, growth occurs in the form of "stalactites".
- Colonies on blood agar are dark brown (absorption of the hemin).
- Colorless colonies (lactose-negative) are formed on MacConkey agar.

BIOCHEMICAL ACTIVITY

- *Y.pestis* ferments glucose, maltose, mannitol but not lactose, sucrose or rhamnose with production of acid only.
- Catalase positive and oxidase negative.
- Urease negative (unlike intestinal yersinia).

ANTIGENIC STRUCTURE

Y.pestis is antigenically homogenous and serotypes do not exist. The antigenic structure is complex. Many antigens have been claimed to be virulence factors. They include following:

- Capsular Ag (F1-Ag) → antiphagocytic effect.
- W-Ag and V-Ag → antiphagocytic effect.
- F2-Ag → exotoxin.
- O-Ag (LPS) → general toxicity.

ECOLOGY AND RESISTANCE

- Yersinia is resistant bacteria in nature (it remains viable for several months in cold, moist environment, soil, rodent burrows).
- Yersinia is obligate pathogen and facultative intracellular parasite of monocytes/macrophages system.

FACTORS OF PATHOGENICITY

1. Capsule (protection against phagocytosis).
2. Intracellular multiplication inside of macrophages (F1, W, V-Ag).
3. Fimbriae (for adhesion).
4. "Plague toxin" consists of endotoxin and "murine exotoxin" (so called because active in rats and mice only, after injection into animals toxin produces edema and necrosis; role of plague toxin in human disease is not known).
5. Extracellular enzymes → coagulase and fibrinolysin (for invasion).
6. Pesticin (bacteriocin which inhibits of *Y.pseudotuberculosis, Y.enterocolitica* and *E.coli*).

CAUSED DISEASES

Clinical forms of **plague**:

(1) Bubonic plague:

• Universally a general lack of energy.

• Fever and intoxication.

• Swelling of lymph nodes resulting in **buboes** (**bubons**), the classic sign of bubonic plague (extremely painful lymphadenitis). The inguinal and axillar lymph nodes are the most frequently affected ("bubon" is Greek for "groin"). From bubon bacilli can enter bloodstream.

(2) Septicemic plague (highly fatal):

• Fever, chills, hypotension, DIC-syndrome is common, shock.

• Purpuric lesions may develop during the systemic stages of infection. These lesions become necrotic and gangrenous and likely explain the name "black death" for the plague.

• Universally a general lack of energy.

• Patient may die before any symptoms appear.

Septicemic plague is usually terminal event in the bubonic or pneumonic plague but it my sometimes occurs primarily.

(3) Pneumonic plague (highly fatal):

• Fever, chills, hypotension, shock.

• Coughing, chest pain, cyanosis, dyspnea (hemorrhagic pneumonia).

EPIDEMIOLOGY

• Source of infection is animals — rodents black rats, grey rats, mice, gophers, marmots (tarbagans) and many others; during pneumonic plague — human beings.

• Mechanisms of transmission are transmissive (bite of rodent fleas), contact with infected rodents and their tissues and inhalation (during person-to-person contact if plague is pulmonary).

There are urban and sylvatic (forest) cycles in epidemiology ofplague.In the sylvatic cycle the rodent is wild, but in the urban cycle, the rodent is domestic. **Epizooty** is epidemy among animals.

There are known 41 epidemics of plague before the birth of Christ and 109 epidemics in next 15 centuries. In the 14th century, pandemic plague was known as the "black death" (due to hemorrhages and gangrene often seen in fatal cases). Now plague survives in several scattered natural foci in some countries of Asia, Africa, America, etc.

IMMUNITY, PREVENTION AND TREATMENT

• Long-lasting immunity.

• Vaccination is for groups of high risk (attenuated EV-vaccine).

• Control of fleas and rodents are of great importance.

• Antibiotics of choice are aminoglycosides (primarily streptomycin).

• Serotherapy with anti-plague gamma-globulin.

Francisella tularensis

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Thiotrichales
Family: Francisellaceae
Genus: **Francisella**
Species: *Francisella tularensis*

MORPHOLOGY

Gram-negative nonsporing **coccobacteria** or **rods**, nonmotile and produce polypeptide capsule.

BIOLOGICAL PROPERTIRES

• Obligate aerobes, capnophiles.

• Mesophiles.

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

- Bacteria with fastidious growth requirements(can't grow on simple media).
- **B**uffered charcoal and **y**east **e**xtract agar (BCYE agar).
- Francis blood dextrose cystine agar.
- Small, transparent S-colonies.
- Biobacteriological method can be used for better cultivation of francisella (infection of mice or guinea pigs with pathological material from patient → inoculation of media with material from animals).

BIOCHEMICAL ACTIVITY

- Francisella ferments glucose and maltose with acids but no gas.
- Catalase positive, oxidase negative.
- H_2S production is marked.

Strains of *F.tularensis* have been subdivided into biotypes based on their virulence, biochemical activity and epidemiology. The most important of those is **type A**, which is found in North America in lagomorphs (rabbits, hares and pikas), and is highly virulent in humans and domestic rabbits. It ferments glycerol. **Type B** occurs mainly in aquatic rodents (beavers, muskrats) in North America and in hares and small rodents in northern Eurasia. It is less virulent for humans and rabbits and lack above mentioned two biochemical properties.

ANTIGENIC STRUCTURE

- O-Ag (LPS).
- K (Vi)-Ag.

ECOLOGY AND RESISTANCE

- Francisella is relatively resistant bacteria in nature.
- Obligate pathogens and facultative intracellular parasites (in macrophages, especially of spleen and liver).

FACTORS OF PATHOGENICITY

1. Capsule (protection against phagocytosis).
2. Fimbriae (for adhesion).
3. Endotoxin (LPS).
4. Extracellular enzymes (for invasion). Francisella are extremely invasive bacteria; they can infect humans even through intact skin.
5. Intracellular persistence in the macrophages.

CAUSED DISEASES

Tularemia (rabbit fever) is named after Tulare County, California.

Depending on the site of infection, tularemia has six characteristic clinical syndromes: ulceroglandular (the most common type representing 75 % of all forms), glandular, oropharyngeal, pneumonic, oculoglandular, and typhoidal.

The clinical signs include fever, lethargy, anorexia, signs of septicemia, and possibly death. The face and eyes redden and become inflamed. Inflammation spreads to the lymph nodes, which enlarge and may suppurate (mimicking bubonic

plague). Lymph node involvement is accompanied by a high fever. Pneumonic form of tularemia is characterized by symptoms of an atypical pneumonia.

EPIDEMIOLOGY

- Source of infection is animals (rodents).
- Mechanisms of transmission are transmissive (bite of ticks, deer flies and several other arthropod vectors), contact with infected rodents and their tissues, inhalation of infected animal wool or dried excreta (no person-to-person contact), fecalo-oral (ingestion of the contaminated food and water by the excreta of the infected rodents).

IMMUNITY, PREVENTION AND TREATMENT

- Long-lasting immunity.
- Vaccination is for groups of high risk (attenuated vaccine).
- Antibiotics of choice are aminoglycosides or tetracyclines.

BACILLI

TAXONOMY

Domain: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Bacillaceae
Genus: **Bacillus**
Species: *Bacillus anthracis*

Anthracoid bacilli are saprophytic and opportunistic members of genus Bacilli which characterized by the next properties: motile, noncapsulated, negative "string of pearls" reaction, rapid liquefaction of gelatin, nonpathogenic for laboratory animals, etc. They can cause PSI (pneumonia, septicemia, meningitis, wound infections, etc.). The most important species is *B.cereus.*

MORPHOLOGY

- **Gram-positive** boxcar-like, large, spore-forming **rods** arranged in short chains (streptobacilli). The ends of the bacilli are truncated or often concave and somewhat swollen so that a chain of bacilli presents a "bamboo stick" appearance.
- Endospores are central and small. They are formed in culture or in soil but never in the animal body during life.
- *B.anthracis*has polypeptide capsule and nonmotile.

BIOLOGICAL PROPERTIRES

- Obligate aerobes and capnophilic; oxygen is required for sporulation, but not for vegetation.
- Mesophiles (optimal temperature for sporulation is 25–30 °C).

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

- *B.anthracis* grows on simple media (MPA, MPB).

• Virulent capsulated strains form R-colonies while avirulent or attenuated strains — S-colonies.

• R-colonies are virulent (large, greyish white, flat, opaque, with a frosted glass appearance). Under a low power of microscope, the edge of the colony is composed of long, interlacing chains of bacilli, resembling locks of matted hair ("Medusa head appearance").

• On gelatin stab culture a characteristic "inverted fir tree" appearance is seen (type of gelatin liquefaction).

• On blood agar colonies are nonhemolytic.

• In broth growth occurs as floccular deposit with little or no turbidity (growth as «cotton wool»).

• PLET-agar (polymyxin, lysozyme, ethylene diamine tetra acetic acid) is selective media used for isolation of *B.anthracis* from mixtures containing other spore-forming bacilli.

• When *B.anthracis* is grown on media containing penicillin the cells of bacilli become large, spherical and occur in chains ("string of pearls" reaction is used for differentiation of *B.anthracis* from *B.cereus*).

BIOCHEMICAL ACTIVITY

Glucose, maltose and sucrose are fermented producing acid but no gas.

ANTIGENIC STRUCTURE

•"Protective antigens" (PA) of exotoxin are protective because antibodies to them block the first step in toxin activity.

•K-Ag (poly-D-glutamic acid).

•Thermoresistant polysaccharide cell wall antigen (provides stability for long periods in tissues obtained from carcasses). The presence of this antigen in animal material is detected by *Ascoli's immune thermoprecipitation reaction*.

ECOLOGY AND RESISTANCE

• The vegetative forms are not particularly resistant. In carcasses of died animals they remain viable in the skin for two weeks.

• The endospores are highly resistant to physical and chemical agents (resist dry heat at 140 °C for 1–3 hours and boiling for 10 minutes). Endospores are retaining in soil, in water for more than 50 years.

• *B.anthracis* is obligate pathogen and extracellular parasite.

FACTORS OF PATHOGENICITY

1. Capsule (protection from phagocytosis).

2. Anthrax exotoxin. It includes three fractions:

• EF — edema factor (factor I).

• PA — protective Ag (factor II).

• LF — lethal factor (factor III).

They are nontoxic individually but the whole complex produces local edema, necrosis and generalized shock. PA is the fraction which binds to the receptors of

the target cells and in turn provides attachment sites for EF and LF. EF is adenyl cyclase which is activated only inside of target cells, leading to the intracellular accumulation of c-AMP and subsequent edema. LF causes cell death (necrosis).

CAUSED DISEASES

Clinical forms of **anthrax** (based on their form of inoculation) are:

(1) Cutaneous anthrax follows entry of infection through the skin (the most common — 95 % of cases): papule → vesicular papule with clear or bloodstained fluid (surrounded area is congested and edematous) → localized, necrotic ulcer (lesion) which is covered by **black eschar** (localization of ulcer is mostly face, neck, hand, forearms, back). The lesion is called **anthrax carbuncle**. The name *anthrax*, which means coal, comes from black color of eschar. Untreated cases may develop fatal septicemia or meningitis.

(2) Pulmonary anthrax ("wool sorter's disease"): highly fatal form is characterized by hemorrhagic pneumonia.

(3) Gastrointestinal anthrax (rare): highly fatal violent enteritis.

EPIDEMIOLOGY

• Source of infection is zoosapronosis (domestic and wild animals, endospores are present in soil).

• Mechanisms of transmission for humans are contact with infected animals and their tissues, inhalation of endospores from animal hair and wool (spores survive long after animal death), fecalo-oral (infected meat). There is no person-to-person spread of pulmonary anthrax.

Animals are infected by ingestion of the spores in soil. Direct spread from animal to animal is rare. The animal disease generally looks like fatal septicemia. Infected animals release bacilli with the different discharges, which sporulate in soil and remain as a source of infection. Animal carcasses are highly infectious.

Anthrax is occupational disease (common in dock workers carrying hides and skins on their bare backs; in workers of wool factories or meat packing, veterinarians, butchers, and farmers).

Anthrax spores can be used as a **biological weapon**.

IMMUNITY, PREVENTION AND TREATMENT

• Long-lasting immunity.

• Vaccination is recommended for animals and persons of high risk (attenuated vaccine with spores of noncapsulated *B. anthracis* stain).

• Prevention: sterilization of animal products; carcasses of animals must be cremated to prevent soil contamination; protective clothing and equipment such as rubber gloves, rubber apron and boots should be used when handling the body; anyone working with anthrax in a suspected or confirmed victim should wear respiratory equipment (respirator).

• Penicillin is the drug of choice for treatment of anthrax; also for serotherapy can be used anthrax antitoxic gamma-globulin.

Bacillus cereus

- Important cause of food poisoning (food toxicoinfection).
- Widely distributed in nature and may be readily isolated from soil, vegetables, variety of foods (milk, cereals, spices, meat and poultry).
- Food poisoning has two patterns caused by different serotypes of *B.cereus*. One is associated with arrange of foods and characteristic by diarrhea and abdominal pain. The second type is associated almost exclusively with consumption of cooked rice, characteristic by acute nausea and vomiting. Both types of disease are mild and self-limiting.
- Isolates from the diarrheal disease produce enterotoxin resembling LT of *E.coli*. Isolates from emetic type of disease can grow only in rice and produce toxin resembling staphylococcal enterotoxin.

OBLIGATELY ANAEROBIC BACTERIA

Medically important obligate anaerobes may be classified as follows:

- **Gram-negative nonsporing rods** (Bacteroides, Prevotella, Fusobacterium, Porphyromonas, Leptotrichia).
- **Gram-positive nonsporing rods** (Eubacterium, Bifidobacterium, Propionibacterium, Lactobacillus, some species of Actinomyces).
- **Gram-positive sporing rods** (Clostridia).
- **Gram-positive cocci** (Peptococci, Peptostreptococci).
- **Gram-negative cocci** (Veillonella).
- Some **Spirochetes**.

GENERAL CHARACTERISTICS OF CLOSTRIDIA

- Taxonomy of Clostridia:
  Domain: Bacteria
  Phylum: Firmicutes
  Class: Clostridia
  Order: Clostridiales
  Family: Clostridiaceae
  Genus: **Clostridium**

- **Gram-positive** anaerobic spore-forming**rods**. The spores are wider than the body of bacteria, giving the bacillus a swollen appearance, resembling a spindle ("*kloster*" means a spindle).
- The spores exhibit a pronounced resistance to heat, drying and disinfectants. Spore formation occurs with varying frequency in different species. Sporulation takes place in the animal body also.
- Localization of spores are using for identification and classification of Clostridia. Spores may be central, subterminal and terminal.
- Clostridia are motile and noncapsulated with exception such as *C.perfringens* (this species is nonmotile and capsulated).
- Types of respiration are **aerotolerant** anaerobes (*C.perfringens, C.histolyticum*) and **obligate** anaerobes (*C.tetani, C.botulinum*, etc.).

• Media for cultivation of the anaerobes: Kitt-Tarozzi medium (MPB + glucose + pieces of liver), Zeissler blood-sugar agar, sugar stab, Wilson-Blair agar (with Na_2SO_3), thioglycollic liquid medium, etc.

• Methods of creation of the anaerobic conditions: special media with reducing agents (fatty acids, ascorbic acid, cystine, thioglycollic acid, glucose, sulphites, etc.), boiling of media and covering by vaseline (oil), anaerostat (anaerobic jar), gas generating boxes.

• Some of the clostridia are normal flora of animal and human intestines (*C.perfringens, C.tetani*). Many species are pathogenic but most are saprophytes found in soil, water and decomposing plant and animal matter.

• The most important clostridioses are **gas gangrene**, **botulism** and **tetanus**.

• In general, clostridia are sensitive to metronidazole, β-lactams, and erythromycin; less to tetracyclines, and resistant to aminoglycosides and quinolones ("natural resistance").

Clostridium perfringens

MORPHOLOGY

Gram-positive nonmotile spore-forming **rods** with polypeptide capsule. It possesses big oval spore of central or subterminal localization.

BIOLOGICAL PROPERTIRES

• Aerotolerant anaerobes.

• Mesophiles.

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

• Milk media → "stormy fermentation".

• Double zone of β-hemolysis on blood agar.

• Wilson-Blair agar → black S-colonies.

• Kitt-Tarozzi medium → homogenous turbidity with gas production.

BIOCHEMICAL ACTIVITY

• Saccharolytic activity: *C.perfringens* ferments glucose, saccharose, lactose producing acid and gas.

• *C.perfringens* produces butyric and acetic acids and large amounts of gases (CO_2, H_2, H_2S, etc.).

ANTIGENIC STRUCTURE

There are 5 serotypes of *C.perfringens*due antigenic variations of produced exotoxins (A-E). Type **A** and more rarely type **C** are pathogenic for humans (table 21).

ECOLOGY AND RESISTANCE

• Colon normal flora of animals and humans; saprophytes.

• Clostridia are relatively resistant in nature but endospores are highly resistant (need for survival of bacteria in the unfavorable conditions).

FACTORS OF PATHOGENICITY

• Factors of adhesion and colonization (fimbria, capsule).

• Capsule of *C.perfringens* (antiphagocytic).

• *C.perfringens* can produce 12 exotoxin with a range of activity: major toxins are α, β, ε, ι; minor toxins are γ, δ, η, θ, κ, λ, μ, ν. There are 5 types of *C.perfringens* (A-E), each type produce a unique spectrum of exotoxins. The clostridial exotoxins show toxic (necrotizing, hemolytic and/or lethal) and invasion activities (collagenase, proteinase, DNAase, lecithinase, hyaluronidase, etc.).

Table 21 — Exotoxins of *C.perfringens* and their role in pathology

Strain of *C.perfringens*	**Toxins types**	**Disease**
Type A	alpha	**Gas gangrene** in animals and humans **Food toxicoinfection**
Type B	alpha, beta, epsilon	Hemorrhagic enteritis in animals
Type C	alpha, beta	**Necrotizing enteritis** in humans Enterotoxenemia in animals
Type D	alpha, epsilon	Enterotoxenemia in sheep
Type E	alpha, iota	Enterotoxenemia in sheep and cattle

The **alpha toxin** is a phospholipase that increases vascular permeability and produces necrosis. **Beta-toxin** and **epsilon-toxin** are membrane pore-forming toxins; **iota-toxin** produces destruction of cytoskeleton. Intoxication by any of these clostridial toxins leads to cell dysfunction and death (necrosis).

• Some strains of *C.perfringens* type A can produce **heat-labile enterotoxins** which resemble enterotoxin of *V.cholerae* and ETEC (→ damage of intestinal epithelium → misbalance of adsorption → fluid accumulation in the intestine resulting in diarrhea).

CAUSED DISEASES

C.perfringens produces the following human infections: gas gangrene, food poisoning, PSI.

❖ **Gas gangrene (clostridial myonecrosis, anaerobic myositis)**: *C.perfringens* type A is the predominant agent. It can be monoinfection, but is more commonly seen in association with other Clostridia (*C.septicum, C.novyi, C.histoliticum,* etc.), nonsporing obligate anaerobes (anaerobic streptococci), facultative anaerobes (staphylococci, *E.coli*), aerobes (*P.aeruginosa*). Gas gangrene is generally disease of war, in which extensive wounds with heavy contamination are so common (spores are present in road dust, soil, bits of clothing, etc.). In civilian life disease follows rod accidents or (rare) surgical operation, injections with lack of asepsis. Clostridia can present on normal skin (peritoneum) and infection may at times be endogenous.

There are several types of **anaerobic wound infection** caused by *C.perfringens*:

(1) Simple wound contamination without invasion of underlying tissue.

(2) Anaerobic cellulitis with minimal toxin production and muscle tissue invasion.

(3) Gas gangreneis associated with severe clostridial invasion of muscle tissue and abundant exotoxins (especially alpha-toxin) production. This results only if the conditionsin a wound are favorable for clostridial multiplication exist (damaged

anoxic muscles). The disease develops with increasing *pain, tenderness, necrosis* and *edema* in the infected site of the body along with systemic sighs of toxenemia (alpha-toxin in blood). Accumulated gases (CO_2 and H_2) make the tissues *crepitant.* There is a watery discharge in wound which later becomes profuse and serous. Profound toxenemia leads to prostration, circulatory shock and death in the untreated cases.

❖ **Food poisoning** (toxicoinfection): usually caused by a cold or warmed up meat dish which is infected clostridial spores. During storage or warming of meat spores germinate; then bacteria multiply and produce enterotoxins in the anaerobic environment of cooked meat. It is self-limiting infection with diarrhea, abdominal pain and vomiting.

❖ **PSI**: gangrenous appendicitis, necrotizing enteritis ("pigbel" caused by *C.perfringens* type C), biliary tract infection, thoratic infections, urogenital infection (following septic abortion), septicemia, etc.

EPIDEMIOLOGY

- Source of infection is sapronosis (soil, road dust, and clothing along with clostridial spores).
- Mechanism of transmission is contact (portal of entry — wounds, burns, disruptions of skin).

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is antitoxic but nonlong-lasting.
- Surgery is the most important and effective measure.
- Antibiotics are effective in prevention before surgical operations.
- Passive immunization with "anti-gas gangrene serum" in view of its uncertain efficacy has become rare.
- Hyperbaric oxygenation therapy and administration of inhibitors of proteolytic enzymes are the additional therapeutic measures for gas gangrene treatment.

Clostridium tetani

MORPHOLOGY

- **Gram-positive** motile noncapsulated spore-forming **rods**.
- It possesses spherical, terminal, large spores, giving the bacillus the characteristic "drumstick" appearance.

BIOLOGICAL PROPERTIRES

- Obligate anaerobes.
- Mesophiles.

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

- Surface colonies are difficult to obtain as the growth has a marked tendency to swarm over the surface of the agar, especially if medium is moist (forming translucent, practically invisible film).
- In deep Wilson-Blair agar the colonies look like spherical fluffy balls.
- Kitt-Tarozzi medium → turbidity with some gas production.
- In blood agar *C.tetani* forms small hemolytic S-colonies.

BIOCHEMICAL ACTIVITY

• *C.tetani* has feeble proteolytic activity.

• It does not attack any sugar (in general biochemically "inert" in comparison with other clostridia).

• H_2S is not formed.

ANTIGENIC STRUCTURE

There are 10 serotypes due to H-Ag that produce the same exotoxin.

ECOLOGY AND RESISTANCE

• *C.tetani* is widely distributed in soil and in the intestines of humans and animals. It is ubiquitous and has been isolated from a wide variety of other sources, including street and hospital dust, cotton wool, bandages, catgut, talc, wall plaster and clothing.

• Clostridia are relatively resistant in nature but endospores are highly resistant (need for survival of bacteria in the unfavorable conditions).

FACTORS OF PATHOGENICITY

C.tetani produces two distinct exotoxins:

• **Tetanolysin** (hemolysin, not relevant in the pathogenesis of tetanus).

• **Tetanospasmin** is powerful neurotoxin which is responsible for tetanus. It is plasmid coded. It gets toxoided spontaneously or in the presence of formaldehyde. It is good antigen and can be specifically neutralized by the antitoxic antisera.

C.tetani has little invasive power. The tetanus exotoxin is produced locally and adsorbed by the motor nerve endings → transport to CNS intraxonally → exotoxin specifically blocks synaptic inhibition in the spinal cord, presumably at inhibitory terminals that use glycine and GABA as neurotransmitters (toxin acts presynaptically) → uncontrolled spread of nerve impulses initiated anywhere in CNS → **muscle rigidity** and **spasms** of trunk and of limbs due to the simultaneous contraction of agonists and antagonists in the absence of reciprocal inhibition ("tetanic" or "tonic" contractions).

CAUSED DISEASES

Tetanus is due to action of exotoxin (tetanospasmin) and characterized by tonic muscular spasms. Most frequently the disease follows injury (puncture wounds are especially vulnerable as they favor the anaerobic conditions). Rare it may follows surgical operations due to lack of asepsis or unsterile injections. Tetanus is important complication of septic abortion. It may be caused by unhygienic practice such as application of cow dung on the umbilical stump or rituals such as ear piercing or circumcision.

Tetanus was a serious disease with high rate of mortality (80–90 %) before specific treatment and prophylaxis is available. Clinical symptoms of tetanus: *trismus* (lockjaw or spasm of masseter muscles), *risus sardonicus* (spasms of mimic muscles), *opisthotonos* (generalized spasms of limbs and trunk).

Tetanus exotoxin can affect neonates to cause muscle spasms, inability to nurse and seizures (neonatal tetanus). Most infants who get the disease die. Immunization of mothers and infants has reduced the incidence of a disease.

EPIDEMIOLOGY

• Source of infection is sapronosis (contamination by spores dressing for wounds, surgical tools and unsterile injectors).

• Mechanism of transmission is contact (portal of entry — puncture wounds, which can be caused by nails, splinters, insect bites, burns, any skin break, and injection-drug sites). Peron-to-person transmission does not occurs at all.

Tetanus is more common in developing countries where the climate is warm, in rural areas where the soil is fertile and highly cultivated.

IMMUNITY, PREVENTION AND TREATMENT

• Immunity is antitoxic but nonlong-lasting.

• For serotherapy (passive immunization) of tetanus high doses of antitoxic human **t**etanus **i**mmuno**g**lobulin (**TIG**) or horse **a**nti**t**etanus **s**erum (**ATS**) are used (test for hypersensitivity is obligatory for ATS).

• Routine active immunization is carried out by DPT (or DTaP, DT). The toxoid is an essential constituent of this vaccine. **DPT** is adsorbed **d**iphtheria, **p**ertussis, **t**etanus vaccine with aluminum hydroxide as adjuvant). Schedule for vaccination is started at the first year of life (at 3, 4, 5 months), than at 18 months and 6 years. Subsequent boosters are injected every 10 years till 66 years (16, 26, 36, etc.).

• Urgent immunization (extra-immunization) is used for persons with trauma:

► Previously vaccinated ("immune") individuals are immunized only with tetanus toxoid (**active** urgent immunization).

► "*Nonimmune*" (have no injection of DPT or immunization status is unknown) patients obtain tetanus toxoid and ATS/TIG (**active-passive** or combined urgent immunization).

• Treatment: anticonvulsive drug therapy, antibiotics, surgical approach, TIG/ATS (patients recovering from tetanus should receive a full course of immunization as a disease does not confer immunity).

Clostridium botulinum

MORPHOLOGY

Gram-positive motile noncapsulated **rods** producing subterminal, oval, large spores (spore-forming cells look like tennis rackets).

BIOLOGICAL PROPERTIRES

• Obligate anaerobes.

• Mesophiles.

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

• Surface colonies are large, irregular, and semitransparent, with fimbriate border (R-colonies) and unpleasant odor of "rancid butter".

• Zeissler sugar-blood agar.

• Kitt-Tarozzi medium → homogenous turbidity.

BIOCHEMICAL ACTIVITY

Biochemical activity varies in different types of *C.botulinum*:

- *C.botulinum* types A, B, F possess both proteolytic and saccharolytic properties but proteolytic are predominating.
- *C.botulinum* types C, D, E possess only saccharolytic properties.

ANTIGENIC STRUCTURE

There are 8 serotypes of *C.botulinum* have been identified (A, B, C1, C2, D, E, F, G) based on the antigenic differences in the *botulotoxin*. The botulotoxin produced by the different serotypes is identical in its pharmacological activity but antigenically different and can be neutralized only by homologous antiserum.

Human disease is usually caused by types A, B, E.

ECOLOGY AND RESISTANCE

- *C.botulinum* is a widely distributed saprophyte, occurring in virgin soil, vegetables, hay, silage, animal manure and sea mud.
- Clostridia are relatively resistant in nature but endospores are highly resistant (need for survival of bacteria in the unfavorable conditions).

FACTORS OF PATHOGENICITY

C.botulinum produces a powerful *neurotoxin exotoxin* (**botulotoxin**) that is responsible for its pathogenicity. In general this bacterium is noninvasive and actually noninfectious.

- The lethal dose of toxin for human is 1–2 μg (it is probably the most toxic substance known).
- The toxin differs from other exotoxins in that it is not released during life of bacteria. It is produced intracellularly and appears only after destruction (autolysis) of the microbe. It is believed to be synthesized initially as a nontoxic prototoxin which is activated under the influence of proteolytic enzymes (trypsin).
- The toxin is relatively heat table (inactivated after 40 minutes at 80 °C and 10 minutes at 100 °C). Toxin production is inhibited in the presence of 6–8 % of NaCl. Also it is resistant to digestive enzymes of GIT.
- Botulotoxin is adsorbed into small intestine and acts by blocking the release of acetylcholine at the synapses and neuromuscular junctions → **cranial nerve involvement** and symmetric descending **flaccid paralysis**. Muscles atrophy but recover in 2–4 months as new terminal axon sprouts form and restore transmission.
- The toxin can be toxoided. It is specifically neutralised by homologous antitoxin and is a good antigen.

CAUSED DISEASES

Botulism is a paralytic disease usually presenting as a form of food poisoning (food intoxication). The name derived from “sausage” (from Latin *botulus*). There are three clinical forms of botulism — foodborne botulism, woundborne botulism and infant (neonatal) botulism.

Foodbornebotulism is due to ingestion of preformed toxin with contaminated food. Symptoms begin usually 12–36 hours after ingestion. Vomiting, thirst, constipation, ocular paresis (*diplopia, mydriasis, ptosis*, etc.), difficulty in

swallowing (*dysphagia*), speaking (*dysarthria*) and breathing constitute the common features. If not treated, the above symptoms may lead to flaccid paralysis of the arms, legs, trunk and lung muscles. Death is due to respiratory failure within 1–7 days. Case fatality varies from 25–70 %.

Woundborne botulism is a very rare condition resulting in wound infection with *C.botulinum* (symptoms are those of foodborne botulism but gastrointestinal components are absent).

Infantbotulism is a *toxicoinfection* (occurs in infant ≤ 6 months). Spores are ingested with food (often honey), get established in the intestine, germinate and produce botulotoxin. The manifestations of infant botulism are constipation, poor feeding, lethargy, weakness, pooled oral secretions, weak cry and loss of head control. Degrees of severity vary from mild illness to fatal disease.

EPIDEMIOLOGY

- Source of infection is sapronosis (contamination of food by spores). Suspected food is usually preserved (meat and meat products, canned vegetables, fish and other seafood, canned mushrooms, poultry, sausages, etc.). Proteolytic types of *C.botulinum* (A, B, F) can digest food, which then appears spoiled (the cans are often inflated and show bubbles on opening). Nonproteolytic types (C, D, and E) leave food unchanged.
- Mechanism of transmission is fecalo-oral (foodborne).

IMMUNITY, PREVENTION AND TREATMENT

- Anti-toxic immunity is very weak.
- Nonspecific prophylaxis is proper canning and preservation of food.
- Passive immunization or serotherapy (should be administered as soon as a clinical diagnosis is made) is carried out with polyvalent/monovalent **botulinum antiserum** or **BIG (botulinumIg)**. Individuals, suspected to use food with botulotoxin, are treated with polyvalent antitoxic sera in lower doses to prevent botulism.
- Adsorbed toxoid can be used for active immunization for person of high risk (laboratory workers).

Clostridium difficile

- It was so named because of difficulty in isolation.
- **Gram-positive** spore-forming **rods**. Spores are large and terminal.
- It is nonhemolytic, saccharolytic and weakly proteolytic.
- *C.difficile* strains are usually resistant to most antibiotics. Metronidazole is the drug of choice. Vancomycin is also useful.
- Caused disease is acute **antibiotic associated colitis** with (**pseudomembranous colitis**) or without membrane formation. Many antibiotics (ampicillin, tetracycline, chloramphenicol, etc.) have been incriminated to provoke this condition.

ANAEROBIC GRAM-POSITIVE COCCI

- **Peptococci** resemble staphylococci in morphology, **peptostreptococci** — streptococci; **veillonella** forms diplococci, short chains or groups.
- They are obligate or aerotolerant anaerobes; grow on blood agar and form small glistering transparent S-colonies.

• Factor of virulence: capsule (antiphagocytic), invasive enzymes (hyaluronidase, collagenase, etc.), exotoxins (hemolysins, cytotoxins).

• Anaerobic cocci are normal inhabitants of the vagina, intestine and mouth.

• They usually cause mixed infections. General type of infections is **PSI** (puerperal sepsis, genital infections, wound infections, UTI, osteomyelitis, abscesses in brain, lungs and other internal organs).

ANAEROBIC GRAM-POSITIVE RODS

• **Eubacteria** are members of the mouth and intestinal normal flora. Some species can cause periodontitis.

• **Propionibacteria** are constantly present in the skin.

• **Lactobacteria** are present in the mouth, intestine and, typically, in the adult vagina (Doderlein's bacilli).

• **Bifidobacteria** are present in the mouth and intestine.

ANAEROBIC GRAM-NEGATIVE RODS

• **Bacteroides** are the most common anaerobes isolated from clinical specimens. It is highly pleomorphic capsulated rods seen singly, in pairs or in short chains. They are obligate anaerobes. They are normal inhabitants of the large intestine. *B.fragilis* is the most frequent of the nonsporing anaerobes.

• **Porphyromonas** genus includes assaccharolytic pigmented species (*P.gingivalis, P.endodontalis*, etc.). They are normal inhabitants of the intestine, mouth/nasopharynx and female genital tract.

• Moderately saccharolytic species inhibited by 20 % bile placed in genus **Prevotella** (*P.melaninogenica, P.denticola*, etc.). They are normal inhabitants of the intestine, mouth/nasopharynx and female genital tract.

• The genus **Fusobacterium** contains long, thin bacilli with pointed ends (*F.nucleatum*). Fusobacteria along with Spirochetes can produce *Vincent's angina* with ulcerative gingivitis. They are normal inhabitants of the intestine and mouth/nasopharynx.

• Factor of virulence: capsule (antiphagocytic), invasive enzymes (hyaluronidase, collagenase, etc.), endotoxin (LPS), exotoxins (hemolysins, cytotoxins).

• **Anaerobic PSI** are usually endogenous infections and generally being provoking by some factors such as trauma, tissue necrosis, impaired circulation or presence of freeing bodies. Anaerobic infections are typically mixed (polyetiological). While the infection is usually localized, general dissemination may occur (bacteremia).

• Examples of anaerobic pyoseptic infections: brain abscess, chronic sinusitis, otitis media, orbital cellulitis, dental abscess, aspiration pneumonia, lung abscess, empyema, hepatic abscess, appendicitis, peritonitis, wound infection after colorectal or genital therapy, puerperal sepsis, tubo-ovarian abscess, septic abortion, breast abscess, axillary abscess, diabetic ulcer, cellulitis, gangrene, etc.

Lecture 15. SPIRAL BACTERIA. RICKETTSIA.CHLAMYDIA. MYCOPLASMA AND UREAPLASMA

GENERAL CHARACTERISTICS OF SPIROCHETES

- Elongated, highly motile, flexible, **helical bacteria** (from *Speira*, meaning coil and *chaite*, meaning hair).
- Characteristic feature is the presence of varying number of **endoflagella** (axial filaments) which are situated between outer membrane and layer of peptidoglycan (in periplasmic space).
- They are Gram-negative bacteria, but Gram staining is not using. The most important methods of detection are Romanovsky-Giemsa staining, impregnation by silver salts (Morozov staining), negative staining with Indian ink (Burry staining), "hanging drop" method for revealing of motility (with dark-field microscopy).
- Many of treponemes are free-living saprophytes, while some of them are obligate parasites.
- Difficult cultivation on the artificial media.
- Taxonomy of spirochetes:

Domain: Bacteria
Phylum: Spirochaetae
Class: Spirochaetes
Order: Spirochaetales

Family: Spirochaetaceae	Family: Leptospiraceae
Genera: **Treponema, Borrelia**	Genus: **Leptospira**

TREPONEMA

Species and subspecies:

- *T.pallidumssp.pallidum* (→ epidemic or venereal syphilis).
- *T.pallidum ssp.endemicum* (→ endemic syphilis).
- *T.pallidum ssp.pertenue* (→ yaws).
- *T.carateum* (→ pinta).

Nonpathogenic treponemes show morphological and antigenic similarities with *T.pallidum*. They occur as commensals in the mouth, intestines and vagina and can be cultivated on artificial media.

MORPHOLOGY

- Treponemes are relatively short **slender spirochetes** with 8–12 regular coils and pointed ends.
- Highly motile (rotation, backward and forward movements, flexion of the whole body.
- Treponema has three endoflagella (axial filaments).
- Outer membrane is rich by lipids (cardiolipin).
- *T.pallidum* is *pale pink* with Romanovsky-Giemsa staining.

BIOLOGICAL PROPERTIRES

• Obligate anaerobes or microaerophiles.

• Mesophiles.

CULTURAL PROPERTIES AND CULTIVATION

• Pathogenic treponemes do not grow on usual artificial media. They can be cultivated in the rabbit testes without lack of their virulence ("tissue treponema"), but this method is long-lasting (3–6 months).

• Treponema can be cultivated on the artificial media enriched with ascitic fluid and brain tissue under anaerobic conditions, but they lose their virulence ("cultural treponema").

BIOCHEMICAL ACTIVITY

• Slow metabolism.

• Catalase and oxidase are negative.

ANTIGENIC STRUCTURE

The antigenic structure of Treponema is complex.

(1) The lipid hapten situated in cell wall is known as **cardiolipin**. It is nonspecific lipid which also can be localized in other host tissues of animals and humans and released during their damage ("antiphospholipid syndrome" during autoimmune diseases, pregnancy, cancer, alcoholism, etc.). Maximal concentration of cardiolipin is characteristic for a bull heart (extraction of bull heart is using as diagnosticum in the **nonspecific tests for syphilis**). Antibodies are formed in response to cardiolipin antigen are called anticardiolipin antibodies (reagin antibodies, reagins). They structurally resemble to the anticardiolipin autoantibodies that are producing as a result of damage of the host tissues. (2) Species specific polysaccharides and proteins: antibodies to these antigens are demonstrated by the **specific treponemal tests** which are positive only with sera of patients infected with pathogenic treponemes.

ECOLOGY AND RESISTANCE

• *T.pallidum* is very sensitive to the factors of environment and disinfectants. Transfusion syphilis can be prevented by storing blood in the refrigerator at least 4 days before transfusion.

• *T.pallidum* is obligate pathogen and facultative intracellular parasite.

FACTORS OF PATHOGENICITY

• Adhesins are synthesizing by treponema only in human organism.

• Lipoproteins take part in immunopathological process but exact role is still unknown.

• Highly invasive bacteria (motility, extracellular enzymes of invasion).

• There is no marked exotoxic and endotoxic activity.

CAUSED DISEASES

Syphilis (lues) is venereal or sexually transmitted disease (STD) which is known for many centuries. There are several periods of syphilis: primary, secondary, latent and late (tertiary, neurosyphilis).

• Primary syphilis. It is usually characterized by an appearance of the hard *chancre* at the site of entry (penis, vagina, anus, nipples, mouth, etc.). In some cases chancre may not be visible (on the uterine cervix). Classical chancre is flat, dull, red, painless, indurated ulcer with serous fluid containing a large number of treponemes (highly infectious). This lesion (ulcer) is known as "hard chancre" to distinguish it from the nonindurated lesion caused by *H.ducreyi* ("soft chancre"). Hard chancre heals spontaneously in 3 to 6 weeks leaving behind a thin scar. The regional lymph nodes are swollen, rubbery and nontender.

• Secondary syphilis (occurs within 6–12 weeks after primary syphilis). The secondary lesions are due to widespread multiplication of spirochetes and their dissemination through blood. Roseolar or papular skin rash (initially appears on the palms and soles and spreads to other areas), mucous patches in oropharynx, condylomas at the mucocutaneous junctions are the clinical manifestations. Spirochetes are detected in lesions; hence the patient is most infectious during this stage. Painless generalized lymphadenopathy is observed. Skin lesions maybe transient and often disappear without treatment, but when the immune state of infected person decreases, the skin lesions appear once again. Duration of secondary syphilis may reach 2–4 years.

• Latent syphilis. There are no any clinical manifestations. It lasts for many years (early latency – less than 4 years, late latency — more than 4 years). In many cases this is followed by natural cure but in others manifestations of late syphilis appear.

• Late granulomatous syphilis. Tissue disintegration is frequent due to hypersensitivity reactions (3 type) or autoimmune pathology. Tertiary lesions ("*gummas*") are granulomatous highly necrotic injuries are localized in skin, mucous membranes, bones, tongue, liver, testes, and aorta (they are hardly infectious or not at all).

There two types of late syphilis based in predominant syndromes: *cardiovascular* syphilis (syphilitic aortitis) and *neurosyphilis.* Neurosyphilis can be differentiated into meningovascular syphilis (obliteration of the small blood vessels in the brain, meninges, spinal cord) and parenchymatous syphilis (destruction of the neurons in the cerebral cortex → paresis or in spinal cord → tabes dorsalis). A great deal of overlap occurs.

Congenital syphilis is syphilis presented*in utero* and at birth, and occurs when a child is born by a mother with syphilis. Untreated early syphilis infections results in a high risk of poor pregnancy outcomes, including saddle nose, lower extremity abnormalities, miscarriage, premature births, stillbirths, or death in neonates. Some infants with congenital syphilis have symptoms at birth, but many develop symptoms later. Newborns will typically not develop a primary chancre, but may present with signs of secondary syphilis (body rash). Often these babies will develop syphilitic rhinitis ("snuffles"). A frequently-found group of symptoms of late congenital syphilis is *Hutchinson's triad* which consists of Hutchinson's teeth (notched incisors), keratitis and deafness.

EPIDEMIOLOGY

- Source of infection is humans.
- Mechanisms of transmission are sexual, transplacental, blood transfusion, direct close contact (with objects of personal utility).

Venereal syphilis is worldwide in distribution.

IMMUNITY, PREVENTION AND TREATMENT

- Immune response is not adequately understood. AMI does not appear to be effective because disease progresses even in the presence of a large amount of Ab. Also autoimmune Ab reactions can provide aggravation of disease by tissue damage. CMI may be more relevant and develops on the basis of DTH (nonsterile immunity). Reinfections do not appear to occur in a person already having active infection and a patient becomes susceptible to reinfection only when original infection is completely eliminated by treatment (however it has been shown some degree of immunity to reinfections).
- Treatment is by antibiotics such as penicillin-G. Dosage and duration of therapy depend of the stage of disease. For local treatment, different ointments with bismuth and mercury are used.
- Prevention is nonspecific. As transmission is by direct contact, it is possible to protect against lues by avoidance of sexual contact with infected individuals. No vaccine is available.

NONVENEREAL TREPONEMATOSES

These diseases occur in endemic foci in several parts of the world, in communities with poor standards of hygiene. Infections are usually transmitted by direct contact. Three distinct forms of endemic treponematoses are recognized – endemic syphilis, yaws and pinta. Causative agents of nonvenenereal treponematoses structurally and antigenically resemble *T.pallidum*.

❖ **Endemic syphilis** (*bejel* in the Middle East, *njovera* in Zimbabwe, *siti* in Gambia). The disease is common in young children. The primary chancre is usually not seen. The disease manifests with mucous patches and skin eruptions which can progress to gummatous lesions.

❖ **Yaws** (also known as *frambesia, pian, parangi*; endemic in tropical areas of Africa, Asia and America). The primary lesion is an extragenital papule which enlarges and breaks down to form an ulcerating granuloma.

❖ **Pinta** (*carate*) is endemic in Central and South America. The primary lesion is an extragenital papule, which does not ulcerate but develops into marked dermal depigmentations.

BORRELIA

Species:

• *B.recurrentis*→ epidemic or louse-borne relapsing fever;

• *B.duttonii, B.hispanica, B.persica*, etc. → endemic or tick-borne relapsing fever;

• *B.burgdorferi* (in USA, Europe), *B.garinii* (in Europe), *B.afzelii* (in Asia)→ Lyme disease (or Lyme borreliosis).

MORPHOLOGY

• Large, **highly motile spirochetes** with irregular, wide 3–8 coils.

• It has 7–11 endoflagella.

• Borrelia is blue-violet with Romanovsky-Giemsa staining.

BIOLOGICAL PROPERTIRES

• Microaerophiles.

• Psychrophiles (optimum temperature for growth is 28–30 °C).

CULTURAL PROPERTIES AND CULTIVATION

• Cultivation is difficult but has been successful in complex media containing serous fluids.

• Growth occurs on the chorioallantoic membrane of chicken embryos.

• The best method for primary isolation is to inoculate mice or guinea pigs intraperitoneally.

BIOCHEMICAL ACTIVITY

• Slow metabolism (nonfermentative bacteria).

• Catalase and oxidase are negative.

ANTIGENIC STRUCTURE

Borrelia has complex antigenic structure:

• Outer surface proteins (Osp A — Osp G).

• H-Ag of endoflagella.

• Heat shock proteins.

Borrelia readily undergoes **antigenic variations***in vivo* (especially, Osp antigens) and this is believed to be a reason for the occurrence of relapses in the disease ("relapsing fever"). Antigen variations have been shown to be caused by DNA rearrangement in circular and linear plasmids which present in Borrelia's genome. Ultimate recovery after a number of relapses may be due to the development of immunity to the all antigenic variants.

ECOLOGY AND RESISTANCE

• Borrelia is sensitive to the factors of environment and disinfectants.

• Borrelia is obligate pathogen and facultative intracellular parasite.

FACTORS OF PATHOGENICITY

• Factors of adhesion.

• Heat shock proteins provoke massive pro-inflammatory cytokine release by host immune cells ("cytokine storm") → tissue damage.

• Endotoxin (LPS) → fever and intoxication.

CAUSED DISEASES

Relapsing fever (RF) is an arthropod-borne infection, two types of which occur — louse-borne and tick-borne. The borrelia causing them is indistinguishable in morphology and many other features but differ in their arthropod hosts.

• After an incubation period RF sets in as fever of sudden onset. During *febrile period* borrelia is abundant in patient's blood. The fever subsides after 3 to

5 days. Then an *afebrile period* around 4 to 10 days occurs (borrelia is not demonstrated in blood) and after that another attack of fever (febrile period) starts again. Borrelia reappears in blood and maybe detected there. The disease subsides after 3 to 10 relapses of fever.

• Splenomegaly is common and jaundice occurs in some cases. In fatal cases, necrotic foci containing borrelia in large number are seen in spleen, liver and other organs. Hemorrhagic lesions are seen in the kidney and intestine. The brain and meninges may also be involved.

• The louse-borne RF is more severe disease characterizing by more severe clinical picture than tick-borne RF which is milder but relapses are more frequent than in louse-borne RF.

EPIDEMIOLOGY

• Epidemic RF used to be very common during wars but with improvement in hygiene and the discovery of insecticides, it has now become rare.

• Louse-borne RF tends to occur as epidemics. Poverty, overcrowding and lack of personal hygiene encourage louse infection. Infection is transmitted not by louse bite but by their being crushed and entered via abraded skin.

Tick-borne RF occurs as sporadic and endemic disease. Transovarial transmission of borrelia in ticks is common.

• Source of infection for epidemic RF is humans; for endemic RF – animals (rodents).

• Mechanism of transmission is transmissive (vectors for epidemic RF are **lice**, for endemic RF are **ticks**).

IMMUNITY, PREVENTION AND TREATMENT

• Immunity after epidemic RF is relatively nonlong-lasting; after endemic RF — relatively long-lasting (especially in the persons from endemic regions).

• Etiological treatment is done by antibiotic such as penicillins, tetracyclines and macrolides.

• There is no specific vaccine.

• Prevention is done by using **insecticides**. Prevention of tick-borne RF is less easy and consists of identification of tick and their avoidance or eradication of the vectors.

Borrelia burgdorferi

• A new spirochetal disease identified in 1975 was named **Lyme disease** (Lyme borreliosis) as it was first observed in Lyme in USA. The disease has subsequently been reported from other parts of the world.

• Clinical picture of Lyme borreliosis includes affection a variety of tissues and organs, including the skin, joints, heart and nervous system.

• Duration of disease classically consists of several stages.

(1 stage) The initial manifestation is expanding annular skin lesion (*erythema migrans*). Primary erythema may reach 40 to 60 sm in diameter. The cen-

ter is pale pink or white while the peripheral area is dark color. Other clinical manifestations are local lymphadenopathy, fever and myalgia.

(2 stage) Hematogenous dissemination of *B.burgdorferi* leads to early disseminated infection with clinical manifestations such as multiple skin lesions (*secondary erythema migrans*), as well as *aseptic meningitis*, *lymphocytic meningoradiculitis Bannwarth, carditis, atrioventricular block* and brief attack of *arthritis*.

(3 stage) Persistent infection (late Lyme borreliosis) occurs months to years after the initial infection and can be associated with *acrodermatitis chronica atrophicans* (diffuse skin rash), chronic *encephalomyelitis* and persistent *arthritis*.

- There are several predominant syndromes associated with certain species of borrelia: Lyme-arthritis is associated with infection of *B. burgdorferi*, neuroborreliosis — *B.garinii* infection, acrodermatitis chronica atrophicans — *B.afzelii*.
- Morphology and cultural properties of Lyme disease pathogens are the same because they are typical borrelia.
- Antigenic structure is complex but not variable as in borrelia causing relapsing fever.
- Factors of pathogenicity: factors of adhesion, Osp-A proteins provide development of immunopathology reactions (ex.: autoimmune arthritis).
- Source of infection is mice, rodents, birds, wild and domestic animals (e.g., deers, cattle, sheep, dogs and many others). Lyme diseaseis transmitted by bite of ticks (transmissive mechanism).
- Treatment is done by antibiotics such as β-lactams and tetracyclines.
- Nonspecific prevention is done by eradication of ticks.

LEPTOSPIRA

Species:

• *L.interoggans* includes pathogenic leptospires. Species further is classified into several serogroups (*Icterohaemorrhagiae, Canicola, Pyrogenes, Australis, Pomona, Grippotyphosa*, etc.). Within each serogroup several serotypes are recognized, for example, the serogroup *Icterohaemorrhagiae* contains the serotypes *icterohaemorrhagiae, smithi, copenhageni*, etc. Over 200 serotypes have been identified and assembled into 22 serogroups. The most virulent serogroup is *icterohaemorrhagiae.*

• *L.biflex*a contains saprophytic leptospires found predominantly in surface waters.

MORPHOLOGY

- Leptospiras are very thin and delicate spirochetes with C- or S-shape of the bacterial body. They possess numerous small coils around 20 to 40. Curves are situated so close together. Their ends are hooked and resemble umbrella handles. They are actively motile.
- Leptospira has 2 endoflagella (axial filaments).
- Leptospira is red with Romanovsky-Giemsa staining. Best methods of de-

tection are dark-field microscopy and silver impregnation method.

BIOLOGICAL PROPERTIRES

• Aerobes and microaerophiles.

• Psychrophiles (optimum temperature for growth is 28–30 °C).

CULTURAL PROPERTIES AND CULTIVATION

• Leptospira can be grown in artificial media enriched with rabbit serum (liquid and semisolid media). They cannot produce visible turbidity and growth must be checked by dark-field microscopy.

• They require prolonged cultivation (several weeks).

• Growth occurs on the chorioallantoic membrane of chicken embryos.

• Inoculation of guinea pigs intraperitoneally is possible.

BIOCHEMICAL ACTIVITY

• Slow metabolism (nonfermentative and nonproteolytic bacteria, use exclusively long-chain fatty acids as the only source of carbon).

• Catalase and oxidase are positive.

ANTIGENIC STRUCTURE

• Leptospiras possess complex antigenic structure.

• Classification into serogroups and serotypes is based on surface antigens (OMPs and O-Ag).

ECOLOGY AND RESISTANCE

• Leptospira is sensitive to the factors of environment and disinfectants but resistant to low temperature.

• Leptospira is widespread in nature. They live in soil and different water reservoirs. Their survival in water or soil depends on temperature, acidity, salinity and other factors.

• *L.interoggans* is obligate pathogen and facultative intracellular parasite.

FACTORS OF PATHOGENICITY

• Factors of adhesion and colonization (→ attachment to the host cells, ex.: renal epithelial cells).

• Endotoxin (→ fever, intoxication and platelet aggregation).

• Exotoxins: hemolysins and cytotoxins.

• Enzymes of invasion: lipase, plasmacoagulase, fibrinolysin, etc.

CAUSED DISEASES

Leptospirosis is actually *generalized vasculitis*. There are no signs of inflammation at the portal of entry. Leptospiras damage mainly the endothelial cells of the capillaries. *Jaundice* is caused by nonnecrotic hepatocellular dysfunction. *Renal failure* results from hypoxic tubular damage. Clinical picture of leptospirosis varies from mild pyrexia to severe or fatal illness with hepatorenal damage.

Leptospirosis is classified into two clinical types — ictericform (severe with jaundice, also known as **Weil's disease**) and anicteric form (milder without

jaundice).

Both types of leptospirosis are characterized by chills, vomiting, abdominal pain, headache, and myalgia. The initial acute stage of disease (first 3–7 days) is called septic (leptospira is seen in the blood). This stage is then followed the second immune stage which lasts 4–30 days. The most important manifestation of second stage of anicteric leptospirosis is *aseptic meningitis*. The second stage of Weil's disease is characterized by hepatic (jaundice), renal (kidney failure) dysfunctions, injection of the eyes ("red eyes") and haemorrhages which occur on skin and mucosa. During late stage leptospiras persist in internal organs mostly in kidney so they can be demonstrated in the urine. The mortality rate of icteric leptospirosis varies within 5–15 %.

EPIDEMIOLOGY

- Source of infection is zoosapronosis (natural reservoir is rodents and field mice in which infection is asymptomatic; they contract infection to domestic animals such as dogs, cattle, pigs and others). Pathogens are excreted with urine and contaminate water and soil.
- Mechanisms of transmission are inhalation of water aerosol (during swimming), fecalo-oral and contact with animals (leptospira invades the human body through microinjuries and intact conjunctival mucosa).

Leptospirosis is occupational disease (persons of high risk are agricultural workers like farmers, fish workers, sewer workers, also veterinarians and others).

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is long-lasting but type specific.
- Treatment is done by antibiotics such as penicillin and tetracyclines. Antibiotic therapy can prevent leptospiruria. Patients with acute renal failure require hemodialysis.
- Vaccination of the domestic animals. Human vaccination is done for high risk groups of people.
- Nonspecific prevention is done by rodent control, disinfection of water and wearing of protected clothes.

Campylobacter

- *C.jejuni, C.coli, C.lari* (found in different animals, especially domestic); *C. fetus* – human opportunistic pathogen.
- **Gram-negative** small spirally **curved bacteria**. They are typically S-shaped rods or multispiral chains. Nonsporing, noncapsulated, very motile with single polar flagellum on one or both ends (monotrichous or amphitrichous).
- O-Ag and H-Ag are present.
- Microaerophiles (10 % O_2), capnophiles (10 % CO_2), thermophiles (42 °C for *C.jejuni*, but for *C.fetus* 25 °C).
- Campylobacters do not attack carbohydrates (they receive energy from amino acids) but are strongly oxidase positive.
- Factors of pathogenicity: factors of adhesion and invasion, endotoxin

(LPS), enterotoxins, cytotoxins.

- Medium for cultivation is blood agar with antibiotics (→ non-hemolytic colonies).
- Source of infection is animals. Mechanism of transmission is fecalo-oral (row milk, meat).
- **Campylobacteriosis** (*C.jejuni*, *C.coli*, *C.lari*) is diarrheal disease (enterocolitis/enteritis, fever, abdominal pain, watery or sometimes bloody diarrhea, but possible extraintestinal PSI such as meningitis, endocarditis, septicemia, etc). *C.fetus* is isolated in cases of PSI in immunocompromised individuals.
- No specific vaccination.

Helicobacter

- *Helicobacter pylori.*
- **Gram-negative** large curved **rods**, highly motile with a unipolar tuft of lophotrichous flagella.
- Microaerophiles, capnophiles, mesophiles.
- Medium for cultivation is chocolate agar.
- It does not ferment carbohydrates, catalase and oxidase are positive.
- Distinctive feature is the production of abundant **urease** (this property has been used as a rapid diagnostic test with gastric biopsy).
- Factors of pathogenicity: pronounced motility; adhesion to the epithelial cells of stomach;**urease** (releases ammonia from urea to facilitate survival of *H.pylori* in a highly acidic environment of a stomach, also causes damage of epiteliocytes); vacuolizing cytotoxin (VacA) that destroys epithelial cells; endotoxin (LPS); enzymes of invasiveness (protease, catalase, lipase).
- Manifestations and clinical forms of **helicobacteriosis**: after incubation period *H.pylori* causes in some person a mild *acute gastritis* which persists for years in most of people. Such infection is usually asymptomatic; the bacteria are present only in the overlying mucous without invasion. Potential sequelae include: *peptic ulcer disease* (duodenum, sometimes gastric ulceration as well) and *chronic atrophic gastritis*. The last infection is recognized as a risk factor for adenocarcinoma and "mucosa associated lymphoid tissue" (MALT) lymphomas.
- Source of infection is humans. Mechanisms of transmission are fecalo-oral and oral-oral (through kissing).
- In patients with ulcers and/or gastric symptoms, a triple combination therapy with omeprazole (proton pump blocker), metronidazole and clarithromycin is recommended.

RICKETTSIA

TAXONOMY

Domain: Bacteria
Phylum: Proteobacteria
Class: Alphaproteobacteria

Order: Rickettsiales

Family: Rickettsiaceae	Family: Anaplasmataceae
Genera: **Rickettsia** and **Orientia**	Genera: **Anaplasma** and **Ehrlichia**
Species: *R.typhi, R.prowazekii, R.rickettsii,* etc. *Orientia tsutsugamushi*	Species: *Anaplasma phagocytophilum, Ehrlichia chaffeensis*

Order: Rhizobiales
Family: Bartonellaceae
Genus: **Bartonella**
Species: *B.henselae, B.quintana,* etc.

MORPHOLOGY

• Small **Gram-negative** pleomorphic **rods/coccobacteria**. They are non-motile and noncapsulated.

• Methods of revealing are Romanovsky-Giemsa staining (bluish purple rickettsia) and Zdradovsky staining (pink rickettsia).

BIOLOGICAL PROPERTIES

• Obligate aerobes.
• Methophiles.

CULTURAL PROPERTIES AND CULTIVATION

• Rickettsia is unable to grow on artificial media.
• They are readily cultivated in the yolk sac of the chicken embryos.
• They also can grow on the cell cultures.
• Laboratory animals such as guinea pig and mice are useful for the isolation of rickettsia from patients. When male guinea pigs are inoculated intraperitoneally they develop fever and a characteristic scrotal inflammation.
• They may also be propagated in arthropods.

BIOCHEMICAL ACTIVITY

Rickettsia metabolism is depending of metabolism of a host cell (this bacterium can't synthesize NAD and lack glycolytic enzymes).

ANTIGENIC STRUCTURE

Rickettsia has species- and group-specific antigens:
• Surface protein antigens (common for rickettsia of typhus group).
• Outer membrane proteins — OMPs (common for rickettsia of spotted fever group).
• Polysaccharide antigen (antigenic mimicry with proteus).

ECOLOGY AND RESISTANCE

• *R.prowazekii* can remain viable and virulent in the dried louse feces for many days.

• **Obligate intracellular parasites** → rickettsia of typhus fever group is localized in the cytoplasm of infected cells. Rickettsia of spotted fever group is

accumulated also in cell nucleus.

- Obligate pathogens.
- Close association with arthropod vectors (ticks, mite and lice).

FACTORS OF PATHOGENICITY

- Factors of adhesion and invasion.
- Endotoxin (LPS).
- Exotoxins (hemolysins).
- Intracellular growth (in cytoplasm or in nucleus).
- Replication of bacteria in vascular endothelial cells → enlargement, degeneration of vascular endothelium and thrombus formation, with partial or complete occlusion of vascular lumen → "vasculitis".

CAUSED DISEASES

Typhus fever group (TG) consists of epidemic typhus, recrudescent typhus (Brill-Zinsser disease) and endemic typhus (table 22).

Table 22 — Diseases caused by Rickettsia and Orientia and their epidemiology

Group	Diseases	Species	SI	MT	Area
Typhus fever group (TG)	Epidemic typhus	*R.prowazekii*	Human	Transmis-sive (lice)	World-wide
	Endemic typhus	*R.typhi*	Rodents (rat)	Transmis-sive (rat fleas)	World-wide Seaports
Spotted fever group (SFG)	Rocky Moun-tain spotted fever (RMSF)	*R.rickettsii*	Rabbit, dog, rodents	Transmis-sive (tick)	Northern America, Canada
	Mediterranean spot-ted fever (MSF)	*R.conori*	Rodents	Transmis-sive (tick)	Mediter-ranean countries
	African tick bite fever	*R. africae*	Rodents	Transmis-sive (tick)	Southern Africa
	Siberian tick typhus	*R.siberica*	Rodents, cattle	Transmis-sive (tick)	Siberia, Far East
	Rickettsial pox	*R.akari*	Mouse	Transmis-sive (tick)	USA, Africa, Russia
	Queensland tick ty-phus	*R.australis*	Rodents	Transmis-sive (tick)	Australia
	Japanese (oriental) spotted fever	*R.japonica*	Rodents, dogs	Transmis-sive (mite)	Japan
	Californian flea rick-ettsiosis	*R.felis*	Opposum	Transmis-sive (fleas)	USA
Scrub typhus group (STG)	Scrub typhus (chigger-borne ty-phus)	*Orientia tsutsugamushi*	Rodents	Transmis-sive (trombicu-lid mite or *chig-gers*)	East Asia, Pa-cific Isl-ands,

					Australia

❖**Epidemic typhus** (Louse-borne typhus, classical typhus) is a form of typhus so named because the disease often causes epidemics following wars and natural disasters. The causative agent is *R.prowazekii*, transmitted by the human body louse. *R.prowazekii* grows in the louse's gut and is excreted in its feces. The disease is then transmitted to an uninfected human who scratches the louse bite (which itches) and rubs the feces into the wound. Symptoms include severe headache, high fever (40 °C), cough, rash, severe myalgia, back pain, chills, photophobia, and mental confusion (stupor)/delirium. A rash begins on the chest and spreads to the trunk and extremities. The name comes from the Greek *typhos* meaning smoky or hazy, describing the state of mind of those affected with typhus (delirium or mental confusion).

Brill-Zinsserdisease is a mild form of epidemic typhus which recurs in someone after a long period of latency. It is endogenous, secondary infection by *R.prowazekii* persisting in monocytes/macrophages (results from reduction of immune protection).

❖ **Endemic typhus** (murine or flea-borne typhus) is milder disease than epidemic typhus. Symptoms include headache, fever, muscle pain, joint pain, nausea and vomiting, possibly skin rash. Up to 45 % will develop neurological signs such as confusion, stupor, seizures.

❖ **Scrub typhus** is caused by *Orientia tsutsugamushi* (from *tsutsuga*, meaning dangerous, and *mushi* meaning insect or mite). Patients typically develop a characteristic eschar at the site of mite bite, with regional lymphadenopathy and maculopapular rash. Encephalitis and pneumonia may be seen in some cases.

EPIDEMIOLOGY

- Source of infection is anthroponosis or zoonosis (table 22).
- Mechanism of transmission is **transmissive** (by arthropod vectors through their bite or feces). Occasionally, epidemic or endemic typhus may also be transmitted through inhalation of dried louse/flea feces or through the conjunctiva. Ingestion of food recently contaminated with infected rat urine or flea feces may also cause endemic typhus.

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is long-lasting, although during epidemic typhus relapse is possible (Brill-Zinsser disease).
- The most effective way to prevent typhus is inoculation with the typhus vaccine series before travelling to endemic areas,and to avoid contact with lice.
- Prompt treatment with doxycycline cures most patients.

Anaplasma

Human granulocytic anaplasmosis (HGA) is an infectious disease caused by *A.phagocytophilum*, an obligate intracellular bacterium that is typically transmitted to humans by at least three kinds of ticks. These ticks also transmit

Lyme borreliosis and other diseases.

Ehrlichia

Human monocytotropic ehrlichiosis (HME) is a form of ehrlichiosis associated with *E.chaffeensis*. The most common symptoms are fever, headache, malaise, and myalgia. The severity of the illness can range from minor or asymptomatic to life-threatening. CNS involvement may occur. A serious septic or toxic shock-like picture can also develop, especially in patients with impaired immunity. This bacterium is an obligate intracellular pathogen affecting monocytes and macrophages.

Bartonella

- Bartonella is a genus of **Gram-negative** bacteria.
- Facultative intracellular parasites, Bartonella can infect healthy people, but are considered as opportunistic pathogens.
- Bartonella species are transmitted by ticks, fleas, sand flies and mosquitoes.
- *B.henselae* is responsible for **cat scratch disease** (CSD), a self-limited disease except in immunocompromised individuals. It is most commonly found in children following a scratch or bite from a cat. Clinical symptoms include regional lymphadenopathy; vesicle or an erythematous papule may form at the site of initial infection.
- *B.bacilliformis* → **Carrion's disease** (Oroya fever, Verruga peruana) endemic for Peru, Ecuador and Colombia.
- *B.quintana* → **trench fever.**

Trench fever (also known as "Five day fever", "Urban trench fever") is a moderately serious disease transmitted by body lice. The disease is classically a five-day fever of the relapsing type, rarely with a continuous course instead. The onset of symptoms is usually sudden with high fever, severe headache, pain on moving the eyeballs, soreness of the muscles of the legs and back, and frequently hyperesthesia of the shins. The most constant symptom is pain in the legs.

- Immunocompromised patients are susceptible to other conditions associated with *B.henselae* and *B.quintana*, such as **bacillary angiomatosis** or **bacillary peliosis**. Bacillary angiomatosis is primarily a vascular skin lesion that may extend to bone or be present in other areas of the body. Bacillary peliosis most often affects patients with HIV. The liver and spleen are primarily affected.

CHLAMYDIA

TAXONOMY	
Domain: Bacteria	
Class: Chlamydiae	
Order: Chlamydiales	
Family: Chlamydiaceae	
Genus: **Chlamydia**	Genus: **Chlamydophila**
Species: *Chlamydia trachomatis*	Species: *Chlamydophila pneumoniae,*

Chlamydophila psittaci

MORPHOLOGY AND REPRODUCTION

• Chlamydia is small **Cram-negative** pleomorphic **cocci** (cell wall lacks peptidoglycan); nonmotile and noncapsulated.

• **Reproductive cycle** is characteristic feature of Chlamydia. Chlamydia occurs in two forms: elementary body and reticulate body.

The **elementary body** (EB) is extracellular and infectious, but metabolically inert (much like a spore). Once the EB attaches to a susceptible epithelial host cell, it mediates its own internalization through type III secretion system that allows for the recruitment of actin with subsequent engulfment of the bacterium. The internalized EB immediately begins differentiation into the **reticulate body** (RB). RBs are metabolically active but non-infectious, and in many regards, resemble normal replicating bacteria (by binary fission). Through unknown mechanisms, RBs begin a differentiation program back to the infectious EBs, which are released from the host cell to initiate a new round of infection. The developing chlamydial microcolony within the host cell is called **inclusion body**. The mature inclusion body contains 100–500 EBs. The reproductive cycle takes 48–72 hours.

• Romanovsky-Giemsa staining (RBs — blue inclusions, EBs — purple).

BIOLOGICAL PROPERTIRES

Obligate aerobes and mesophiles.

CULTURAL PROPERTIES AND CULTIVATION

• Chlamydia is unable to grow in artificial media.

• They are readily cultivated on the cell cultures.

• They also can grow in the yolk sac of the chicken embryo and laboratory animals such as mice.

BIOCHEMICAL ACTIVITY

Chlamydial metabolism is depending of metabolism of a host cell ("**energy parasites**"), so they have weak biochemical activity.

ANTIGENIC STRUCTURE

Chlamydia possesses several kinds of antigens: (1) genus-specific Ag common to all Chlamydia (LPS); (2) species-specific protein Ag in cell wall; (3) major outer membrane proteins (MOMP) which are type-specific (found only in some members of species).

C.trachomatis includes 18 serotypes that cause certain infections (table 23): A, B, C serotypes → eye chlamydiosis, D-K serotypes → genital chlamydiosis and extragenital complications, serotypes L1–L3 → venereal lymphogranulomatosis.

ECOLOGY AND RESISTANCE

• **Obligate intracellular parasites** (they lack enzymes of electron transport chain and so require ATP and nutrition from host cell – "*energy parasites*"). *C.trachomatis* infects epithelial cell, *C.pneumoniae* – alveolar macrophages, monocytes and endothelial cells. *C.psittaci* can affect different cell including

monocytes/macrophages.

- Obligate pathogens.
- Chlamydia is not resistant in nature.

FACTORS OF PATHOGENICITY

- Factors of adhesion and colonization (OMPs).
- Endotoxin (LPS).
- Exotoxins (cytotoxins).
- Heat-sock proteins (HSPs) → induction of autoimmune pathology.

CAUSED DISEASES

❖ **Trachoma** is a chronic follicular *keratoconjunctivitis* characterized by inflammation of the conjunctiva and cornea which leads to scarring and blindness. Trachoma is a common cause of blindness worldwide.

❖ **Inclusion conjunctivitis** in adult is follicular hypertrophy with scanty nonpurulent discharge (also known as "*swimming pool conjunctivitis*" due to its association with bathing in swimming pools contaminated with genital secretions). Contamination of eye with patient's own genital secretions may be the cause more often. "*Inclusion blenorrhea*" is neonatal form of inclusion conjunctivitis develops when the infant is infected in birth passage.

❖ **LGV** is STD characterized by suppurative inguinal adenitis endemic for tropical countries.

❖ **Genital chlamydiosis** is most common STD worldwide. Its clinical picture is parallel to gonococcal infection.

- In men clinical forms are acute *urethritis* ("nongonococcal urethritis — NGU), *epididymitis, proctitis, conjunctivitis* and *Reiter's syndrome* (trial of recurrent conjunctivitis, arthritis and urethritis). Clinical sighs of urethritis are nonpurulent discharge from the penis and burning during urination. Untreated chlamydiosis in men can lead to dissemination of infection and even to infertility.
- Clinical forms in women are acute *urethritis, bartholinitis, cervicitis, endometritis, salpingitis, pelvic inflammatory disease* (PID), *conjunctivitis* and *Reiter's syndrome*.

Genital chlamydiosis may lead to infertility, ectopic pregnancy, premature delivery, perinatal morbidity (sepsis, pneumonia, meningoencephalitis of newborns) and postpartum fever. Also genital chlamydiosis is known as "silent" infection because of ~75 % of the infected women and ~50 % of the infected men have no symptoms. However, if women have symptoms they include lower abdominal pain, fever, pain during sex, urination, urge to urinate more frequently than usual (urinary urgency). Women are often reinfected if their sex partner is not treated.

❖ **Psittacosis** varies from mild flu-like syndrome to fatal pneumonia, can develop septicemia, meningoencephalitis, endocarditis, and arthritis or typhoid-like syndrome.

❖ **Chlamydia pneumonia** is caused by strain TWAR (from **T**ai**w**an **A**cute **R**espiratory) of *C.pneumoniae* and appears to be a common reason of respiratory diseases in older children and adults. Its clinical spectrum includes pharyngitis,

sinusitis, bronchitis and pneumonia.

Table 23 — Human diseases caused by chlamydia and chlamydophila

Genus	Species	Biotype	Serotypes	Diseases
Chlamydia	*C.trachomatis*	Trachoma	A, B, C	Trachoma
			D-K	Inclusion conjunctivitis (neonatal and adult) Genital chlamydiosis Infant pneumonia
		LGV	L1, L2, L3	Lymphogranuloma venerium (LGV)
Chlamydophila	*C.pneumoniae*	TWAR	-	Acute respiratory diseases
	C.psittaci	-	8 sero-types	Psittacosis

EPIDEMIOLOGY

- *Trachoma*: source of infection is humans, mechanisms of transmission are eye-to-eye contact by fingers, towels, dust particles; indirect contact (mechanical transfer of infection with flies). This disease is prevalent in developing countries because of overcrowding and unhygienic conditions; it is endemic for Middle East, Africa, India and Far East.
- *Genital chlamydiosis*: source of infection is humans, mechanisms of transmission are sexual, close direct contact (through the personal utensils and bedclothes), passage through the birth canal.
- *Psittacosis*: source of infection is parrots, turkeys and other birds. Human infections are mostly occupational (poultry workers). Mechanism of transmission is inhalation during contact with birds.
- *Chlamydia pneumonia*: source of infection is humans; mechanism of transmission is inhalation (sneezing, cough).

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is nonlong-lasting in the most of infections (after LGV is long-lasting; during psittacosis — nonsterile CMI on the base of DTH and repeated infections are possible).
- Vaccines against chlamydial infections are not available.
- Prevention: improved sanitary conditions and safe sexual contacts.
- Tetracyclines (e.g., doxycycline) and macrolides are commonly used antibiotics. Erythromycin is given to pregnant women.
- Immunomodulators can be used (IFN preparations).

MYCOPLASMA

TAXONOMY

Domain: Bacteria
Phylum: Tenericutes or Firmicutes
Class: Mollicutes
Order: Mycoplasmatales
Family: Mycoplasmataceae

Genus: **Mycoplasma**
Species: *M.genitalium, M.hominis,*

Genus: **Ureaplasma**
Species: *U.urealyticum*

M.pnemoniae, M.fermetans, etc.

MORPHOLOGY

- Mycoplasma is a group of bacteria that lack cell wall and so are **highly pleomorphic**, with no fixed shape (*Myco*, from the fungus-like form of branching filaments; *plasma*, denoting their plasticity of shape). They occur as granules (coccoid, discs, and ring or star forms) and filaments of various sizes.
- They are nonmotile and noncapsulated; considered as Gram-negative bacteria, but staining causes them to disintegrate.
- The cells are bounded by a soft trilaminar membrane containing sterols (they are "**membrane parasites**").
- Method of revealing is phase-contrast or dark-field microscopy.

BIOLOGICAL PROPERTIRES

- Facultative anaerobes.
- Mesophiles.

CULTURAL PROPERTIES AND MEDIA FOR CULTIVATION

- Mycoplasmashould be cultivated in special isotonic fluid or solid media enriched with horse or human serum. Penicillin and thallium acetate are added as selective agents.
- The colony is typically biphasic, with a "fried egg" appearance, consisting of a central opaque granular area of growth extending into the depth of the medium, surrounded by a flat, translucent peripheral zone (they are very small and can be seen with a hand lens).
- Can be readily cultivated in the cell cultures and chicken embryos (on the chorioallantoic membrane).

BIOCHEMICAL ACTIVITY

- Mycoplasmas possess a fermentative metabolism. The most of species ferment glucose or arginine as the major source of energy.
- Mycoplasma requires cholesterol and related sterols as components of its cell membrane (it is feature is unique among prokaryotes).

ANTIGENIC STRUCTURE

- Mycoplasmal surface antigens are mainly glycolipids and proteins.
- Useful technique for the identification of isolates is the **growth inhibition test** based on the ability of specific antisera to specifically inhibit the growth of the homologous species.

ECOLOGY AND RESISTANCE

- Mycoplasma may be saprophytic (smallest free-living bacteria) and parasitic (obligate and facultative pathogens). Parasitic species are "membrane parasites".
- Mycoplasmas are widely distributed (soil, water) but generally not resistant in nature. *M.hominis* (and *U.urealyticum*) occurs in healthy person as a part of normal flora (so their etiological role has to be confirmed by quantitative content $\rightarrow \geq 10^4$).
- They are relatively resistant to lysis by osmotic shock but very sensitive to the surface active agents (taurocholate).
- They are ***resistant to β-lactams*** and lysozyme that act on the bacterial cell

wall but sensitive to tetracyclines and macrolides.

FACTORS OF PATHOGENICITY

1. Adhesion factors (proteins P1 of *M.pneumoniae*) → cessation of ciliary movement → disturbance of the normal clearance mechanisms in RT → contamination of RT → development of a dry cough.

2. Toxic metabolic products (H_2O_2 and superoxide) → accumulation and damage of the host membranes → close fusion of mycoplasmas with a damaged host membrane during adhesion.

3. Toxins: endotoxin (LPS), hemolysins → various cell destructions.

4. Enzymes of invasion (phospholipase, protease, nuclease, etc.).

5. Immunopathogenesis → mycoplasma can affect macrophages and stimulate cytokine production and lymphocytes activation → autoimmune complications.

CAUSED DISEASES

M.pneumoniae → respiratory mycoplasmosis (upper respiratory tract infection, tracheobronchitis, atypical pneumonia).

M.hominis and *M.genitalium* → genital mycoplasmosis.

M.fermetans → infections in HIV-positive individuals.

❖ **Mycoplasmal pneumonia** (primary atypical pneumonia, "walking pneumonia") is characterized with paucity of respiratory signs on physical examination but radiological evidence of consolidation, which is usually patchy, involving one of the lower lobes. The onset is gradual, with fever, malaise, headache and sore throat, later paroxysmal persistent cough is developing. The disease is usually self-limited but can be prolonged. The complications are otitis, meningitis, encephalitis, arthritis, rash and hemolytic anemia.

❖ **Genital mycoplasmosis** includes urethritis, proctitis, Reiter's syndrome in men and acute salpingitis, PID, cervicitis, vaginitis in women. They also have been associated with infertility, abortion, postpartum fever, and postnatal pathology.

EPIDEMIOLOGY

- Source of infection is human.
- Mechanism of infection is inhalation (spread is facilitated by close contact, as in families and military recruits) for respiratory mycoplasmosis and sexual for genital mycoplasmosis (also passage through the birth canal).

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is nonlong-lasting.
- Vaccines against mycoplasmal infections are not available.
- Prevention: safe sexual contacts for genital mycoplasmosis.
- Tetracycline and erythromycin are the drugs of choice for the treatment of mycoplasmal infection.

Ureaplasma urealyticum

- They are peculiar in their ability to hydrolyze **urea**, which is essential factor in addition to cholesterol.
- Formation of very small and tiny colonies.
- *U.urealyticum* produces **genital ureaplasmosis** (clinically resemble ge-

nital mycoplasmosis).

Lecture 16. GENERAL VIROLOGY

GENERAL PROPERTIES OF VIRUSES

A **virus** is a small infectious agent that replicates only inside the living cells of other organisms ("smallest living units"). Viruses can infect all types of life forms, from humans, animals and plants to bacteria.

Viruses are characterized by the following properties:

- Viruses do not possess cellular organization and contain only one type of nucleic acid, either DNA or RNA but never both.
- They are **obligate intracellular parasites.**
- Viruses do not have their own metabolism (lack enzymes necessary for protein and nucleic acid synthesis and are dependent for replication on the synthetic machinery of a host cell).
- They reproduce by creating multiple copies of themselves through self-assembly ("disjunctive reproduction").
- Viral DNA can be integrated into the DNA of the infected cell ("virogenia"); integrated virus DNA is called **provirus**.
- They are unaffected by the antibacterial antibiotics.
- They inherit genetic mutations.

Viroid is protein-free subviral agent with very small genome (low molecular weight RNA). Viroids have been shown to cause plant diseases. **Prion** is unconventional proteinaceous infectious virus-like agent without any detectable nucleic acid responsible for slow infections of humans and animals (scrapie, Kuru, Cruetzfeldt-Jacob disease, etc.).

MORPHOLOGY

Viruses are much smaller than bacteria. They are called "**ultramicroscopic**" as they are too small to be seen under a light microscopy. The most direct method of measuring virus size is **e**lectron **m**icroscopy (optical, scanning and transmission EM). Most viruses that have been studied have a diameter between 20 and 300 nanometers. Some filoviruses have a total length of up to 1400 nm.

The extracellular infectious virus particle is called the **virion**. The virion consists of a nucleic acid (genome) surrounded by a protein coat, the **capsid**. The capsid with the enclosed nucleic acid is called **nucleocapsid**. The capsid is composed of a large number symmetrically arranged **capsomers** (subunits). The function of the capsid is to protect nucleic acid from inactivation by nucleases and other factors.

There are two kinds of capsid symmetry – cubic (icosahedral) and helical. Some viruses, like poxvirus exhibits a complex symmetry.

Virions may enveloped and nonenveloped (naked). **Envelope** or **supercapsid** (outer covering of viruses) is lipoprotein coat of **complex viruses** derived from the host cell membrane when the progeny virus is released by budding. The envelope lipids are of host cell origin while proteins/glycoproteins (p/gp) are virus coded. Proteins/glycoproteins of envelope are also called **spikes**. Ex.:

hemagglutinins are surface gp of hemagglutinating viruses which is capable for agglutination various types of human and bird/animal erythrocytes. **Simple** (nonenveloped, naked) **viruses** have no envelope. Functions of envelope are protection, adsorption and penetration of virus, antigenic, shaping of virus.

In several virus species enzymes are a component of the virus particle. Ex.: *neuraminidase* of ortho- and paramyxoviruses (need for invasion and release of viruses); RT (*reverse transcriptase*) of retroviruses.

The overall shape of the virus particle varies in different groups of viruses. Most animal and human viruses are spherical. Some are pleomorphic. The rabies virus is bullet shaped. Ebola virus is filamentous virus. Poxviruses are brick shaped.

VIRAL GENOMES

A virus has either a DNA or an RNA genome and is called a DNA virus or an RNA virus, respectively. The vast majority of viruses have RNA genomes. A viral genome, irrespective of nucleic acid type, is almost always either single-stranded (ss) or double-stranded (ds).

Table 24 — Genomic diversity among viruses

Property	Parameters
Nucleic acid	DNA RNA
Shape	Linear Circular Segmented/nonsegmented
Strandedness	Single-stranded (ss) Double-stranded (ds) Double-stranded with regions of single-strandedness
Sense	Positive sense (+) Negative sense (−)

For the most of viruses with RNA genome, the single strands are said to be either **positive-sense** or **negative-sense**, depending on if they are complementary to the viral mRNA. +ssRNA is in the same sense as viral mRNA and thus at least a part of it can be immediately translated to viral proteins by the host cell. -ssRNA is complementary to mRNA and thus must be converted to mRNA before translation.

RESISTENCE

- Viruses are very heat labile (they are inactivated at 56 °C within seconds and at 37 °C within minutes).
- A better method for storage is lyophilisation (freeze storage).
- Viruses are inactivated by sunlight, UV rays and ionizing radiations.
- They are, in general, more resistant than bacteria to chemical disinfectants. The most active antiviral disinfectants are oxidizing agents such as H_2H_2, $KMnO_4$ and hypochlorites. Chlorination of drinking water kills most viruses but its efficacy is greatly influenced by the presence of organic matter. Some viruses

(hepatitis virus, enteroviruses) are relatively resistant to chlorination.

• The action of lipids solvents (ether, chloroform, bile salts) is selective (enveloped viruses are sensitive and naked viruses are resistant).

• Antibacterial antibiotics are completely ineffective against viruses. This property is used for eliminating of bacteria from clinical specimens before virus isolation.

CLASSIFICATION OF VIRUSES

Criteria for virus classification:

✓ Type and organization of viral genome.

✓ The strategy of viral replication.

✓ The structure of virion.

✓ Natural host range and mode of transmission.

✓ Cell and tissue tropism.

✓ Pathogenicity and cytopathology.

✓ Physicochemical properties of the virion, including molecular mass, pH stability, thermal stability, and susceptibility to physical and chemical agents, especially ether and detergents.

✓ Virus protein properties, including number, size, and functional activities of structural and nonstructural proteins, modifications (glycosylation, phosphorylation), and special functional activities (transcriptase, reverse transcriptase, neuraminidase, fusion activities).

✓ Antigenic properties.

The **International Committee on Taxonomy of Viruses** (ICTV) developed the current classification system of viruses. The general taxonomic structure is as follows:

Order (-virales)

Family (-viridae)

Subfamily (-virinae)

Genus (-virus)

Species (-virus)

The committee does not formally distinguish between subspecies, strains, and isolates. In total there are 7 orders, 103 families, 22 subfamilies, 455 genera, about 2,827 species.

Specie may include serogroups, subtypes, serotypes, isolates (strains).

For example, family *Picornaviridae* → genus *Enterovirus* → species *Poliovirus (PV)* → serotypes *Poliovirus 1*, *2*, and *3* → strains *PV-1 "Mahoney"* or PV-3 *Leon/37*.

Baltimore classification

The Baltimore Classification of viruses is based on the method of viral mRNA synthesis. The ICTV classification system is used in conjunction with the Baltimore classification system in modern virus classification.

Viruses must generate mRNAs from their genomes to produce proteins and

replicate themselves, but different mechanisms are used to achieve this in each virus family. Viral genomes may be **ss** or **ds**, RNA or DNA; some viruses can use reverse transcriptase (RT). In addition, ssRNA viruses may be either sense (+) or antisense (−).

This classification places viruses into seven groups:

I: **dsDNA** viruses (e.g. Adenoviruses, Herpesviruses, Poxviruses)

II: **ssDNA** viruses (e.g. Parvoviruses)

III: **dsRNA** viruses (e.g. Reoviruses)

IV: **+ssRNA** viruses (e.g. Picornaviruses, Togaviruses)

V: **−ssRNA** viruses (e.g. Orthomyxoviruses, Rhabdoviruses)

VI: **ssRNA-RT** viruses with DNA intermediate in life-cycle (e.g. Retroviruses)

VII: **dsDNA-RT** viruses (e.g. Hepadnaviruses).

Table 25 — DNA viruses

Family	Type of DNA	Virion	Capsid
Parvoviridae **Circinoviridae**	ssDNA	simple	cubic
Papillomaviridae **Polyomaviridae** **Adenoviridae**	dsDNA	simple	cubic
Hepadnaviridae	dsDNA with ss-fragment	complex	cubic
Herpesviridae	dsDNA	complex	cubic
Poxviridae	dsDNA	complex	complex

Table 26 — RNA viruses

Family	Type of RNA	Virion	Capsid
Picornaviridae **Astroviridae** **Caliciviridae**	+ssRNA	simple	cubic
Reoviridae	dsRNA segmented	simple	cubic
Togaviridae **Flaviviridae**	+ssRNA	complex	cubic
Coronaviridae	+ssRNA	complex	helical
Retroviridae	+ssRNA diploid genome	complex	cubic (cone-like shape)
Orthomyxoviridae **Arenaviridae** **Bunyaviridae**	-ssRNA segmented	complex	helical
Paramyxoviridae **Rhabdoviridae** **Filoviridae**	-ssRNA	complex	helical

VIRAL MULTIPLICATION

There are six basic, overlapping stages in the life cycle of viruses in living cells:

(1) **Adsorption** (attachment) is the binding of the virus to specific molecules (receptors) on the surface of the cell. This specificity restricts the virus to a very limited type of cell. For example, the human immunodeficiency virus

(HIV) infects only human T-cells, because its surface protein, gp120, can only react with CD4. In case of influenza virus the hemagglutinin (H) on the virus get attached to glycoprotein receptors on the surface of respiratory epithelium. So differences in susceptibility to viral infections are to a large extent based on the presence or absence of receptors on the host cells.

(2) **Penetration** follows attachment; viruses penetrate the host cell by endocytosis ("viropexis") or by fusion with the cell (viral envelope fuses with plasma membrane of the host cell and release the nucleocapsid into the cytoplasm).

(3) **Uncoating** happens inside the cell when the viral capsid is removed and destroyed by viral/host enzymes for release of the viral nucleic acid ("stripping" of a virus).

(4) **Biosynthesis** (replication) includes synthesis of virus proteins and virus genomes by the host cell. Most DNA viruses synthesize their nucleic acids in the host cell nucleus. Most RNA viruses synthesize their components in the host cell cytoplasm. Viral proteins are synthesized only in the cytoplasm. Biosynthesis consists of following steps:

a) Transcription of viral mRNA from the viral nucleic acid.

b) Translation of mRNA into "early proteins" or nonstructural proteins which are enzymes for initiating and maintaining synthesis of viral components.

c) Replication of viral nucleic acid.

d) Synthesis of "late proteins" or structural (components of capsid and envelope).

The critical step is transcription of mRNA; depending on the structure of their genome viruses use different strategies for transcription for certain group due to Baltimore classification. Examples:

dsDNA → mRNA → proteins

ssDNA → dsDNA → mRNA → proteins

+ssRNA → proteins

-ssRNA → mRNA → proteins

ssRNA-RT → DNA → mRNA → proteins.

(5) **Assembly** takes place in the cell when the newly created viral proteins and nucleic acid combine to form hundreds of new virus particles ("viral progeny"). At this stage simple viruses are present intracellularly as fully developed virions. In case of complex viruses only nucleocapsids are complete. Envelopes derived from the host cell membrane during the process of budding. The host cell membrane which becomes the envelope is modified by incorporation of virus-specific glycoproteins.

(6) **Release** occurs when the new viruses escape or are released from the cell. Most viruses achieve this by making the cells burst, a process called lysis. Other viruses such as HIV are released more gently by a process called budding (exocytosis).

VIRAL GENETICS

The two main mechanisms for genetic modification of viruses are mutation

and recombination.

● **Mutation** may occur spontaneously or may be induced by mutagens (irradiation, chemical agents, etc.). The frequency of mutation in viruses is about the same as in bacteria (10^{-4}–10^{-8}). Most of these point mutations are "silent" — they do not change the protein that the gene encodes — but others can confer evolutionary advantages such as resistance to antiviral drugs.

Medically important are mutants with weakened virulence that have retained their antigenicity intact — "*attenuated viruses*" (material of alive vaccines).

● **Recombination** may occur when two different, but related, viruses infect a cell simultaneously. These viruses exchange segments of genome between them so that hybrid results (ex.: antigenic shift in influenza viruses). Recombination may take place between the virus and host cell genome (virogenia) that leads top changes of the host cell such as *malignant transformation*.

● **Nongenetic interactions:** in mixed infections viral components can be exchanged or they may complement (or interfere with) each other's functions (phenotypic mixing, complementation, interference).

● **"Quasispecies":** it is heterogeneous population from all of the possible mutants of a viral species → high level of virus variability (HIV, hepatitis C virus) → escape of the host immune response.

CULTIVATION OF VIRUSES

As viruses are obligate intracellular parasites they can be cultivated on any artificial culture media. Three methods are employed for the virus cultivation – chicken embryos, cell cultures and laboratory animals.

Chicken embryo (embryonated egg) offers several methods of inoculation and cultivation of viruses: inoculation on chorioallantoic membrane (CAM), inoculation into allantoic or amniotic cavities, yolk sac inoculation.

Cell culture is a technique for maintaining or growing cells in vitro. Cultures of dispersed cells derived directly from fresh tissues are called primary cell cultures. They are capable of only limited growth in culture and cannot be maintain in serial culture. Common examples are monkey kidney, human embryotic kidney, human amnion and chicken embryo cell cultures. Primary cell cultures are useful for the isolation of viruses and their cultivation for vaccine production. Diploid cell cultures retain original diploid chromosome number during serial cultivation but about 50 serial passages, they undergo "senescence". Examples are human fibroblasts, human embryonic lung, and Rhesus embryo cell cultures. They are also employed for the production of viral vaccines. Continuous cell culture is cells of a single type, usually derived from cancer cells that are capable of continuous serial cultivation indefinitely. Examples are HeLa (human carcinoma of cervix), HEP-2 (human epithelioma of larynx), McCoy (human synovial carcinoma), Detroit-6 (sternal marrow), Vero (velvet monkey kidney), BHK-21 (baby hamster kidney) cell cultures.

Laboratory animals (monkey, white mice) are used for cultivation and identification of virus, also for study of pathogenesis, immune response, epide-

miology and oncogenesis.

CONSEQUENSES OF VIRAL INFECTION FOR THE HOST CELL

- **Cytocidal** or **cytolytic infection (necrosis)**: viral replication results directly in cell destruction/lysis (cytopathology or CPE — **c**yto**p**athic **e**ffect in cell culture).
- **Apoptosis**: virus induces cell "suicide".
- **Noncytocidal (noncytolytic) infection:** viral replication does not produce cell destruction, although it may be destroyed by immunological reactions (with help of NK-cells, CTLs).
- **Latent infection** (there is neither viral replication nor cell destruction): virus is "dormant" (in state of provirus or episomes) → *latency* or *persistence* (protects viruses from immune system activity and thus is a strategy of virus survival). However a variety of factors can initiate a lytic cycle leading to manifest disease and dissemination of virus (recidivation or reactivation).
- **Abortive infection** may result when a virus mistakenly infects a cell that does not permit viral replication.
- **Tumor transformation** (oncogenic viruses such as retroviruses, herpes viruses, hepatitis B virus, etc.).

VIRAL PATHOGENESIS

- **Tropism** is ability of viruses to infect only certain cell types due the presence of specific receptors on the surface of cells (enterotropic, neurotropic, lymphotropic, cardiotropic, hepatotropic viruses).
- **Viral dissemination in the organism**: some viruses can cause only *localized infections* but many of them spread further from the portal of entry via blood ("viremia/virusemia") or along nerves and produce *generalized (disseminated) infections*.
- **Persistence**: it is long-term survival of a virus inside of the host cell. Mechanisms of persistence: (a) escaping of immune system by constant mutations (ex.: HIV); (b) down regulating of the host immune system (ex.: cytomegalovirus codes proteins that reduce the expression of MHC class I on the cell surface); (c) integration of the viral genome into the host genome (ex.: HIV, hepatitis B virus).
- **Course of disease**: depending on the clinical outcome viral infections can be classified as *apparent* (clinical) and *inapparent* (subclinical). They also may be *acute, subacute* and *persistent (latent* or *chronic).* An *acute* viral infection is characterized by rapid onset of disease, a relatively brief period of symptoms and resolution within days (recovery or death). *Persistent infection* is long-term infection. *Chronic* infection is referring to a long-term condition with periods of relapses and remissions. *Latent infection* is long-term infection without any clinical symptoms.

HOST RESPONSES TO VIRUS INFECTION

- **Nonspecific immune defense**: (a) the most important humoral antiviral factor is interferon (IFN) → synthesis of "antiviral" proteins → inhibition of viral replication. (b) NK-cells and K-cells provide nonspecific killing of the vi-

rus-infected cells.

• **Specific immune defense:** includes CMI and AMI. In the most of viral infections CMI is more important than AMI. The cytotoxic T-lymphocytes (CTLs) are capable to recognize and destroy the virus-infected cells on the surface of which viral antigens are expressed. The humoral immunity can eliminate only extracellular viruses. IgG and IgM play a major role in blood and tissue spaces, sIgA is more important in mucosal surfaces. Some Ab can paradoxically enhance viral infectivity ("Ab enhancement" phenomenon). Also Ab may cause immediate type of hypersensitivity — II type of ITH (complement-dependent injury of cells) or III type of ITH (immune complex hypersensitivity).

PREVENTION

Prolonged and effective immunity is a characteristic of the most of viral infections. Viral vaccines (**active immunization**) also confer solid protection and are, in general, more effective than bacterial vaccines. *Killed (inactivated) vaccines* generally provide short-lived immunity and weaker immunity than *attenuated (alive) vaccines*. Other types of vaccines are recombinant, DNA-, subcellular/subunit vaccines, etc.

The most important vaccine-preventable viral infections are poliomyelitis, rabies, yellow fever, varicella, measles, mumps, rubella, influenza, hepatitis B and A.

Passive immunization with human Ig is only used in small amount of cases, usually as postexposure prophylaxis. The protection conferred is of short duration and only effect against viruses causing viremia.

CHEMOTHERAPY

Inhibitors of certain steps of viral replication can be used as **chemotherapeutic agents** to treat viral infections. As viruses are obligate intracellular parasites it is may not be possible to inhibit viral replication without damage of the host cells. However, there are several areas available for attack of the virus selectively. Viral infection can be stopped at the level of adsorption, transcription of viral mRNA, replication of viral genomes, assembly and release of viral progeny. A number of virus-specific enzymes have been identified which can be inhibited selectively.

Available antiviral agents can be divided into the following categories:

• **Nucleoside analogues**: these analogues of thymidine, adenine and guanine: antiherpetic preparation — *Acyclovir*; anti-HIV preparations (*Azidothymidine, Lamivudine and Stavudine*); *Ribavirine* (against many RNA and DNA viruses).

• **Other drugs**: *Amantadine* (blocks penetration of influenza A virus), *Foscarnet* (inhibits DNA polymerase of herpes viruses), etc.

• **IFN**: beneficial effect has been observed in persistent infections such as hepatitis B and C, herpes, laryngeal papilloma, etc.

In spite of intensive efforts, progress in the field of antiviral chemotherapy has not been satisfactory. The available drugs have a narrow range of activity. Viruses also can develop *resistance to the drugs* and break through infection

takes place even during treatment.

EPIDEMIOLOGY

The major means of transmission may be by droplet or aerosol mode (influenza, measles); by the fecal-oral route (enteroviruses, rotaviruses, infectious hepatitis A); by sexual contact (hepatitis B virus, genital herpes virus, HIV); by hand-mouth, hand-eye or mouth-mouth contact (herpes viruses, rhinovirus, Epstein-Barr virus); or by exchange of contaminated blood (hepatitis B virus, HIV). Spread may be by bite (rabies) or by droplet or aerosol infection from rodent-contaminated quarters (arenaviruses); also transmission by means of arthropod vectors (togaviruses, flaviviruses and bunyaviruses), etc.

EMERGING VIRAL INFECTIONS

Viral diseases emerge following one of three general patterns: recognition of a new agent, abrupt increase in illnesses caused by an endemic agent and invasion of a new host population.

Combinations of factors contribute to disease emergence. Factors include (1) environmental changes (deforestation, damming, flood or drought); (2) human behavior (sexual behavior, drug use); (3) socioeconomic and demographic phenomena (war, poverty, population growth and migration, urban decay); (4) travel (highways, international air travel); (5) food production (changes in methods of food processing and packaging); (6) health care (new medical devices, blood transfusions, organ transplantation, drugs causing immunosuppression, widespread use of antibiotics); (7) microbial adaptation (changes in virulence, development of drug resistance, cofactors in chronic diseases); and (8) public health measures (inadequate sanitation and vector control measures, curtailment of prevention programs). Examples of emerging viral infections in different regions of the world include Ebola virus, Nipah virus, hantavirus pulmonary disease, HIV infection, dengue hemorrhagic fever, West Nile virus, Rift Valley fever, and bovine spongiform encephalopathy (prion disease), etc.

Lecture 17. VIRUSES ARE CAUSATIVE AGENTS OF ACUTE RESPIRATORY VIRAL INFECTIONS

The most common acute viral infections are probably upper and lower respiratory tract infections (**ARVI** — **a**cute **r**espiratory **v**iral **i**nfections). Although these infections can be classified by the causative virus (table 26), they are generally classified clinically according to syndrome (ex.: the common cold, AFRD, coryza, bronchitis, croup, pneumonia, etc.).

- **AFRD** is **a**cute **f**ebrile **r**espiratory **d**isease.
- **Common cold** (upper respiratory tract infection/URT) is acute, usually afebrile, self-limited infection with rhinitis, cough and sore throat.
- **Coryza (rhinitis)** is irritation and inflammation of the mucous membrane inside the nose.
- **Bronchitis** is an inflammation of the mucous membranes of the bronchi. **Bronchiolitis** is inflammation of the bronchioles.
- **Pneumonia** is an inflammation of the lung affecting primarily the alveoli (typical symptoms include a cough, chest pain, fever, and difficulty breathing).
- **Croup** (**laryngotracheobronchitis**) is a respiratory condition that leads to swelling inside the throat, which interferes with normal breathing and produces the classical symptoms of a "barking" cough, stridor and hoarseness (in small children may be airway obstruction).
- **Influenza-like illness**, also known as **a**cute **r**espiratory **i**nfection (ARI) and flu-like syndrome, is a medical diagnosis of *possible influenza* or other illness causing a set of *common symptoms* (fever, shivering, chills, malaise, dry cough, loss of appetite, body aches and nausea, typically in connection with a sudden onset of illness).

Although specific pathogens commonly cause characteristic clinical manifestations (ex.: rhinovirus typically causes the common cold, RSV typically causes bronchiolitis, etc.), each can cause many of the viral respiratory syndromes.

Severity of ARVI varies widely; severe disease is more likely in the elderly and infants. Morbidity may results directly from viral infection or may be indirect, due to exacerbation of underlying cardiopulmonary conditions or bacterial superinfection of the lungs, paranasal sinusitis or middle ear.

Treatment of ARVI is usually supportive. Antibiotics should be given only when secondary bacterial infections develop.

Other than ARVI infectious diseases can have respiratory syndromes include malaria, acute HIV infection, herpes, Lyme disease, rabies, Q fever, dengue fever, poliomyelitis and many others.

Table 27 — Characteristics of the causative agents of ARVI

Family	Common viruses as agents of ARVI	Caused infection and principal syndromes
Orthomyxoviridae	Influenza A, B and C viruses	*Seasonal influenza* (AFRD, common cold, acute bronchitis and pneumonia) *Avian influenza* *Swine influenza*
Paramyxoviridae	Parainfluenza viruses	*Parainfluenza* (AFRD, common cold, laryngitis, acute bronchitis, pneumonia, croup)
	RSV (respiratory-syncytial virus)	*RS-infection* (bronchiolitis in infants and mild common cold in adults)
	Human metapneumovirus	Infection resembles RS-infection
	Mumps virus	*Mumps*
	Measles virus	*Measles*
Togaviridae	Rubella virus	*Rubella*
Adenoviridae	Adenoviruses	*Adenoviral infection* (AFRD, acute pharyngoconjunctival fever, pneumonia)
Picornaviridae	Rhinoviruses	*Rhinoviral infection* (common cold, acute coryza with/or without fever)
	Enteroviruses and Parechoviruses	*Enteroviral infection* (common cold) *Herpangina*
Parvoviridae	Bocaviruses	*Bocaviral infection* (bronchitis, pneumonia and common cold in children)
Coronaviruses	Common coronaviruses (CoV) SARS-CoV MERS-CoV	*Coronaviral infection* *SARS* *MERS*
Herpesviridae	HSV-1 (herpes simplex virus type 1)	*Nasopharyngitis*
	EBV (Epstein-Barr virus), CMV (cytomegalovirus)	*Infectious mononucleosis* *CMV-pneumonia*
	VZV (varicella-zoster virus)	*Chickenpox*

ORTHOMYXOVIRUSES

CLASSIFICATION

Group (Baltimore classification): Group V (-ssRNA)
Order: Unassigned
Family: Orthomyxoviridae
Genus: Influenzavirus A → Species (type): **Influenza A virus**
Genus: Influenzavirus B → Species (type): **Influenza B virus**

Genus: Influenzavirus C → Species (type): **Influenza C virus**

MORPHOLOGY

• Influenza virus is typically spherical with size 80-120 nm but pleomorphism is common.

• **Complex** (enveloped) viruses with **helical** symmetry of capsid. Envelope has 2 types of surface gp (spikes): **hemagglutinin** and **neuraminidase**.

• **Hemagglutination** is important characteristic influenza viruses.

• The **negative** sense **single stranded RNA** genome (-ssRNA) is segmented (8 segments for Influenza A and B viruses and 7 segments for Influenza C virus).

ANTIGENIC STRUCTURE

The antigens of influenza virus can be classified as internal and surface antigens. The internal Ag is **ribonucleoprotein** (RNP). It is type-specific and based on its nature, influenza viruses are classified into types A, B and C. RNP-Ag is stable and does not exhibit any significant antigenic variation. **M–Ag** (matrix) is also type-specific.

Surface antigens are hemagglutinin (H) and neuraminidase (N).

• **Hemagglutinin (H)**: it is glycoprotein which is responsible for hemagglutination and hemoadsorption. It enables the virus to adsorb to mucoprotein (sialic) receptors of the respiratory epithelium cells. Antibodies against **H** are protective (prevent adsorption of a virus to cells). **H** is type-specific Ag is capable of *great antigenic variation.* 16 distinct subtypes (**H1**–**H16**) have been identified for avian and human influenza viruses.

• **Neuraminidase (N)**: it is glycoprotein enzyme which destroys cell sialic receptors by hydrolytic cleavage. Antibodies are not so effective in protection like antihemagglutinin Ab. **N** is also type-specific Ag and capable of *antigenic variation.* 9 distinct subtypes (**N1**-**N9**) have been identified for avian and human influenza viruses.

Antigenic variation of influenza virus

Antigenic variability is highest in influenza A virus, less in type B, while it has not been demonstrated in type C. There are two types of antigenic variations — antigenic shift and antigenic drift.

Antigenic shift is abrupt, discontinuous variation in the antigenic structure, resulting in novel virus strain (formation new subtype). The mechanism for shift is *genetic reassortment* (recombination) between human and avian influenza viruses (possible another animal reservoir). Shift is characteristic only for influenza A virus; types B and C viruses do not exhibit antigenic shift because of few related viruses exist in animals. Shift occurs at infrequent intervals (10–40 years the last century); as a result, the population has no immunity to the new virus and pandemic influenza may occur.

Antigenic drift is due to the *accumulation of point mutations* in the gene,

resulting in aminoacid changes in the proteins (H and N). A strain must sustain two or more mutations before a new, epidemiologically significant strain (serotype) emerges (may lead to epidemics about every 2–3 years).

Specific influenza strain isolates are identified by a standard nomenclature specifying virus type, geographical location where virus first isolated, sequential number of isolate, year of isolation and subtype (H_N_).

Examples of the nomenclature are: A/Brisbane/59/2007 (H1N1), A/Moscow/10/99 (H3N2).

Table 28 — Differential characteristics of influenza viruses types

Feature	Influenza A virus	Influenza B virus	Influenza C virus
Hemagglutinin and Neuraminidase	H1-H16 N1-N9	H N	H -
Segments of RNA	8	8	7
Antigenic shift	+++	-	-
Antigenic drift	+++	++	-/+
Subtypes	H1N1, H2N2, H3N2, H5N1, H7N7, H7N9 and others	absent	absent
Strains	Many (Singapore, Hong Cong, Brisbane, Moscow etc)	Many (Victoria, Yamagata)	In a small amount
Virulence	+++	++	+
Natural reservoir	Human, pig, bird, horse	Human (rare seal)	Human (rare dogs and pigs)
Spreading	**Pandemics**, epidemics, local outbreaks	Epidemics, local outbreaks	Local outbreaks and sporadic cases

The type A viruses are the most virulent human pathogens among the three influenza types and cause the most severe disease. The serotypes that have been confirmed in humans, ordered by the number of known human pandemic deaths, are:

- ✓ H1N1 caused "Spanish Flu" in 1918, "Swine flu" in 2009.
- ✓ H2N2 caused "Asian Flu".
- ✓ H3N2 caused "Hong Kong Flu".
- ✓ H5N1 is a pandemic threat (avian flu).
- ✓ H7N7 has unusual zoonotic potential.
- ✓ H1N2 is endemic in humans and pigs.
- ✓ H9N2, H7N2, H7N3, H10N7.

The most common and widely distributed subtypes which are circulated in human population are **H1N1, H2N2** and **H3N2**.

CULTIVATION

Classically, embryonated eggs (chicken embryos) and primary monkey kidney cell culture have been the isolation methods of choice for influenza viruses. Cell cultures can be tested for the presence of virus by **hemadsorption**,

while chicken embryo — by **hemagglutination**.

CAUSED DISEASES

Influenza (flu, grippe, grip) is a well-known acute viral respiratory infection (ARVI) causing fever, rhinitis (coryza), cough, headache (especially in back and legs) and malaise. Headache is prominent, often with photophobia.

Influenza causes cellular destruction and desquamation of superficial mucosa of RT but do not affect the basal layer of epithelium. Viral damage to the respiratory tract epithelium lowers its resistance to secondary bacterial invaders, especially staphylococci, streptococci, and *H.influenzae*. The prominent systemic symptoms associated with the production of cytokines.

Mild (common cold) or asymptomatic infections may occur. Mortality is possible during seasonal epidemics, particularly among high-risk patients (children < 4 yr, adults > 65 yr, pregnant women, etc.)

Clinical symptoms of influenza in children are similar to those in adults, although children may have higher fever and a higher incidence of gastrointestinal manifestations such as vomiting. Febrile convulsions can occur. Influenza A viruses are an important cause of croup in children under 1 year of age, which may be severe.

When influenza appears in epidemic form, clinical findings are consistent enough that the disease can be diagnosed. Sporadic cases cannot be diagnosed on clinical symptoms, as disease manifestations cannot be distinguished from those caused by other ARVI.

Pneumonia complicating influenza can be viral, secondary bacterial or combination of the two. *S.aureus* coinfection has been reported to have a fatality rate of up to 42 %.

Other complications of influenza: encephalitis, myocarditis, renal failure. *Reye's syndrome* is an acute encephalopathy and "fatty liver" syndrome of children and adolescents (mortality rate is 10–40 %). The cause of Reye's syndrome is unknown, but it is a recognized rare complication of influenza B and A (possible relationship between aspirin/salicylate use and subsequent development of Reye's syndrome).

EPIDEMIOLOGY

- Source of infection is zooanthroponosis.
- Influenza virus spreads from person to person by airborne droplets or by contact with contaminated hands or surfaces.

IMMUNITY, PREVENTION AND TREATMENT

- Immunity to influenza is long-lived and subtype-specific. Immunity can be incomplete, as reinfection with the same virus can occur. The three types of influenza viruses are antigenically unrelated and therefore induce no cross-protection. When a viral type undergoes antigenic drift, a person with preexisting antibodies to the original strain may suffer only mild infection with the new strain.
- Vaccines are composed of virions of the H1N1 and H3N2 human influenza A viruses, as well as those of influenza B viruses. Vaccines are reformulated annually by updating the seed strains. Type of vaccines: inactivated, attenuated, sub-

virion (include purified virus components) and subunit vaccines (consist of different combinations of purified hemagglutinin and neuraminidase glycoproteins).

- Nonspecific prophylaxis includes staying at home, air sterilization, rooms ventilating and disinfection, also masks, washing of hands, etc.
- Drugs available for the treatment of influenza include adamantanes (*Amantadine* and *Rimantadine*), which inhibits the uncoating of virions by interfering with M protein, and *oseltamivir* (brand name "*Tamiflu*"), which inhibits the viral neuraminidase.

H1N1 Swine influenza (pandemic H1N1/09 virus)

- The 2009 flu pandemic or **swine flu** was an influenza pandemic and the second of the two pandemics involving H1N1 influenza virus (the first of them being the 1918 flu pandemic), albeit in a new version.
- Before being transmitted to humans, H1N1 type virus is known to have circulated in swine. First described in April 2009, the virus appeared to be a new strain of H1N1 which resulted when a previous triple reassortment of bird, swine and human flu viruses further combined with a Eurasian pig flu virus, leading to the term "*swine flu*".
- The virus is contagious and is believed to spread from human to human. The infection is not acquired by ingestion of pork and is acquired very rarely by contact with infected pigs.
- Symptoms and complications resemble those of ordinary influenza, although *gastrointestinal symptoms* (nausea, vomiting and diarrhea) are common. Symptoms are usually mild, but they can become severe, leading to pneumonia or respiratory failure. H1N1/09 destroys the lungs' alveoli, often causing ***a**cute **r**espiratory **d**istress **s**yndrome* (ARDS), which kills in half of all cases.
- Antivirals (oseltamivir, zanamivir) were recommended for those with more severe symptoms or those in an at-risk group. Vaccines for **H1N1pdm** infection have been developed.

Avian influenza (bird flu)

- Avian influenza — influenza endemic to pigeons and some birds.
- Low-pathogenic viruses cause few or no symptoms in infected birds. However, some strains can mutate into *highly pathogenic avian influenza* (HPAI) strains that are extremely infectious and deadly to birds. Avian viruses can infect pigs, but people are generally not affected. That changed when there was an outbreak of **H5N1** in Hong Kong in 1997 (Influenza A virus subtype H5N1). Influenza A virus subtype **H7N9**, first reported in China in February 2013. Spread to humans was contained by culling domestic bird's populations. Human infections with other avian influenza strains have also been reported in Asia (**H9N2**), Canada (**H7N3**) and Netherlands (**H7N7**).
- All influenza viruses are capable of rapid genetic change, raising the possibility that avian strains could acquire the ability to spread more easily from

person to person via direct mutation (drift) or via recombination with human strains or porcine host (shift). Many experts are concerned that if these strains acquire the ability to spread efficiently from person to person, an influenza pandemic could result.

• People experienced traditional flu symptoms such as a fever, cough, sore throat, and aching muscles. Other symptoms included conjunctivitis, pneumonia, acute respiratory distress syndrome, viral pneumonia, and other severe and life-threatening complications.

• H5N1 is resistant to adamantanes; resistance to oseltamivir has also been reported.

PARAMYXOVIRUSES

CLASSIFICATION

Group (Baltimore classification): Group V (-ssRNA)

Order: Mononegavirales

Family: Paramyxoviridae

Subfamily: Paramyxovirinae

Genus: Henipavirus → Species: **Hendra virus** and **Nipah virus** (**henipa**viruses are recently recognized zoonotic viral diseases naturally harbored by fruit bats (flying foxes) in Australia, Malaysia, Singapore and Bangladesh and associated with *encephalitis* in humans).

Genus: Morbillivirus → Species: **measles virus**

Genus: Respirovirus → Species: human **parainfluenza virus** (1 and 3 types) and **Sendai virus** (Sendai virus is zoonotic virus but rare can cause URTs in humans).

Genus: Rubulavirus → Species: human **parainfluenza virus** (2 and 4 types) and **mumps virus**

Subfamily: Pneumovirinae

Genus: Metapneumovirus → Species: **human metapneumovirus**

Genus: Pneumovirus → Species: **respiratory syncytial virus** (RSV)

MORPHOLOGY

• Paramyxoviruses resemble orthomyxoviruses in morphology but they are larger (100–300 nm) and more pleomorphic. They are **complex** viruses with **helical** symmetry of capsid.

• The **negative** sense **single stranded RNA** genome (-ssRNA) is nonsegmented (hence all paramyxoviruses are antigenically stable).

ANTIGENIC STRUCTURE

Envelope has two types of gp spikes on the surface.

• The longer spike is hemagglutinin (H) which may also possess neuraminidase (N) activity and is hence known as **H-Ag** or **HN-Ag**. It is responsible for adsorption of the virus to the host cell surface.

• The second spike is F (fusion) protein (**F-Ag**), responsible for fusion of the viral envelope with the plasma membrane of the host cells. It also brings

about cell-to-cell fusion, causing CPE (cytopathic effect) in cell cultures such as symplasts or syncytia (large giant cells). Also F-Ag displays hemolysin activity.

Table 29 — Properties of paramyxoviruses

Property	Parainfluenza viruses	Mumps virus	Measles virus	RSV
Antigens	NH-Ag F-Ag	NH-Ag F-Ag	H-Ag F-Ag	G-Ag F-Ag
Serotypes	4	1	1	1
CPE	**symplast** formation	**symplast** formation and cytoplasmic **inclusions**	**symplast** formation and nuclear/ cytoplasmic **inclusions**	**syncytium** formation

CULTIVATION

• Classically, primary monkey kidney cell culture, HeLa, Vero cell cultures have been the isolation method of choice for paramyxoviruses viruses. Cell cultures can be tested for the presence of viruses by **hemadsorption** and indication of the cytopathic effect (CPE) such as **symplasts/syncytium** formation and/or presence of the **viral inclusions**.

• Chicken embryos can be used for cultivation of mumps virus (method of indication is **hemagglutination**).

CAUSED DISEASES

❖ **Mumps (epidemic parotitis)** is acute respiratory infection commonly affecting children and characteristic with painful enlargement of the salivary glands, most commonly the parotids. The virus replicates in URT and cervical lymph nodes and is disseminated through the bloodstream to various organs (testes, pancreas, thyroid glands, CNS, etc.). Orchitis is a complication seen in ~30 % of postpubertal boys (→ testicular atrophy or sterility). Mumps meningitis/meningoencephalitis, pancreatitis, oophoritis, myocarditis have been reported.

❖ **Measles (rubeola)** is acute respiratory infection commonly affecting children and characteristic with specific rash. A day or two before rash appears *Koplik's spots* develop on the buccal mucosa (small white ulcerations contain symplasts and viral inclusions). The red maculopapular rash typically appears on the forehead first and spreads downwards, to disappear in the same manner 3–6 days later, leaving behind a brownish discoloration and desquamation. The rash represents an immune reaction between T-cells and virus-infecting vascular endothelium cells.

Pathogenesis of measles: the virus replicates in URT and regional lymph nodes → primary viremia → multiplication in monocytes/macrophages → secondary viremia → replication of virus in the epithelial surfaces including the skin, mouth, RT and conjunctiva. The common complications are viral croup/bronchitis, meningoencephalitis and secondary bacterial infections (pneumonia, otitis media). A rare late complication is **s**ubacute **s**clerosing **p**an**e**ncephalitis (**SSPE**).

❖ **RS-infection** is first isolated from chimpanzees with coryza ("chimpanzee coryza agent"). Now it is the most important cause of lower respiratory tract infection in infants, particularly in the first few months of life (bronchiolitis,

pneumonia). In adults RS-infection presents as common cold or rare pneumonia.

❖ **Parainfluenza** is acute respiratory infection of children and adults. In children the most serious clinical syndrome is croup which is most frequently caused by types 1 and 2; type 3 causes lower respiratory tract infections such as bronchitis and pneumonia; type 4 causes minor respiratory illnesses. In adults parainfluenza represents as mild respiratory infection in which sore throat and hoarseness of voice are common.

❖ **Metapneumovirus infection** may be the second most common cause (after RSV) of lower respiratory infection in young children (bronchitis, bronchiolitis and pneumonia). Compared with RSV, infection with human metapneumovirus tends to occur in slightly older children and to produce disease that is less severe. Co-infection with both viruses can occur, and is generally associated with worse disease.

EPIDEMIOLOGY

• Source of infection is anthroponosis for most of the infections.

• *Mumps* is transmitted by direct contact, airborne droplets or subjects contaminated with saliva (toys). *Measles* is highly contagious infection (infectivity is maximal at the prodrome and diminishes rapidly with onset of the rash). Spread is by direct contact with respiratory secretion (coughing, sneezing) and airborne droplets. *RS-infection* and parainfluenza are transmitted by close contact (contaminated fingers) and airborne droplets.

IMMUNITY, PREVENTION AND TREATMENT

• Immunity after mumps and measles is long-lasting. Reinfection with RSV is not uncommon but the disease so produced is milder than primary infection. Parainfluenza viruses share cross-reactivity but tend to cause diseases of different severity (type 4 has antigenic cross-reactivity with mumps and appears to be an uncommon cause of ARVI). First infections are more serious than reinfections, which are not infrequent. With type 4 virus, even first infections are very mild.

• It is recommended that this vaccine be given as measles-mumps-rubella (MMR) vaccine at 12 months of age and a second dose at 4-6 years of age (prior to school entry). No effective vaccines against parainfluenza, RS-infection are available.

• Normal human gammaglobulin can prevent measles.

CORONAVIRUSES

CLASSIFICATION

Group (Baltimore classification): Group IV (+ssRNA)
Order: Nidovirales
Family: Coronaviridae
Subfamily: Coronavirinae
Genus: Alphacoronavirus
→ Species: human **coronaviruses 229E** and **NL63** (ex.: HCoV-229E)
Genus: Betacoronavirus
→ Species: human **coronaviruses OC43** and **HKU1**
→ Species: **M**iddle **E**ast **r**espiratory **s**yndrome coronavirus (**MERSCoV**)
→ Species: **s**evere **a**cute **r**espiratory **s**yndrome virus (**SARS-CoV**)
Genus: Deltacoronavirus

Genus: Gammacoronavirus → Species: avian coronavirus
Subfamily: Torovirinae
Genus: Torovirus → Species: animal toroviruses

MORPHOLOGY

• Coronaviruses are **enveloped viruses** with a **positive-sense RNA genome** and with a nucleocapsid of **helical symmetry**.

• The name "coronavirus" is derived from the Latin *corona*, meaning crown, and refers to the characteristic appearance of virions under electron microscopy with a fringe of large, bulbous surface projections creating an image of the solar corona (this morphology is created by the viral spikes).

ANTIGENIC STRUCTURE

Proteins that contribute to the overall structure of all coronaviruses are the spike (S), envelope (E), membrane (M) and nucleocapsid (N). In the specific case of the SARS coronavirus, a defined receptor-binding domain on S mediates the attachment of the virus to its cellular receptor, angiotensin-converting enzyme 2 (ACE2).

CULTIVATION

Coronaviruses can be cultivated in the cell cultures but they are difficult to replicate in the laboratory.

CAUSED DISEASES

Coronaviruses are believed to cause a significant percentage of all common colds in human adults. Coronaviruses also cause a range of diseases in farm animals and domesticated pets, some of which can be serious and are a threat to the farming industry.

❖ **HCoV-229E** and **HCoV-OC43** are associated with a range of respiratory symptoms, ranging from the common cold to high-morbidity outcomes such as pneumonia and bronchitis.

❖ **HCoV-NL63** is a novel virus that was identified in 2003 in a child with bronchiolitis in the Netherlands. Now virus spreads worldwide and is found mainly in young children, elderly and immunocompromised patients with acute respiratory illness during the winter season. Recent data suggest an association of HCoV-NL63 infection with *Kawasaki disease* (it is the most common cause of acquired heart disease in children in developing countries).

❖ **HCoV-HKU1** was first identified in January, 2005, in a 71-year-old man who was hospitalized with an acute respiratory distress. The virus was identified in some cases of pneumonia.

❖ **SARS coronavirus** (SARS-CoV) is much more severe than other coronaviral infections. This virus was identified in 2003 in Asia. SARS-CoV has a unique pathogenesis because it causes both upper and lower respiratory tract infections and can also cause gastroenteritis. The symptoms include high fever, chills, muscle aches, headaches, *diarrhea* (large volume and watery, usually in the second week of illness), sore throat, runny nose, malaise, myalgia, dry cough and shortness of breath. At that time, a chest x-ray is ordered to confirm pneumonia. In severe cases, it develops into respiratory failure and ARDS.

❖ **MERS-CoV** was first reported in 2012 in Saudi Arabia. Reservoir is unknown (possible a bat). MERS is transmissible from human-to-human. Symptoms include fever, chills, muscle aches, cough (gastrointestinal symptoms less characteristic than in SARS and occur in ~30 % of patients). Chest x-ray is also ordered to confirm pneumonia. A few patients have acute kidney failure.

EPIDEMIOLOGY

- Source of infection is anthroponosis for the most of the infections. The particular animal species cannot be excluded as the possible sources for SARS and MERS.
- Mechanism of transmission is mainly air borne, possible direct contact and oral-to-oral (for SARS).

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is studied not enough but it is likely type-specific.
- Treatment is symptomatic; vaccines are not available.

ADENOVIRUSES

CLASSIFICATION

Group: Group I (dsDNA)
Order: Unassigned
Family: Adenoviridae
Genus: Mastadenovirus (including all human adenoviruses); other genera include avian, ovine, frog adenoviruses and others
Species: **Human adenovirus** (groups A-G)

MORPHOLOGY

- Adenoviruses are medium-sized (90–100 nm), **simple viruses** with **cubic capsid** containing a **double stranded DNA** genome.
- Their name derives from their initial isolation from human *adenoids*.
- The virion also has a unique "spikes" or **fibers** associated with capsid that helps in attachment to the host cells.

ANTIGENIC STRUCTURE

In humans, there are 57 accepted human adenovirus serotypes in seven groups (human adenovirus A-G):

A → 12, 18, 31
B → 3, 7, 11, 14, 16, 21, 34, 35, 50, 55
C → 1, 2, 5, 6, 57
D → 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 36, 37, 38, 39, 42, 43, 44, 45, 46, 47, 48, 49, 51, 53, 54, 56
E → 4
F → 40, 41
G → 52

Type-specific antigens are located on fibers and identified by neutralization test. Different types/serotypes are associated with different clinical conditions: respiratory disease (mainly species B and C); conjunctivitis (B and D); and gastroenteritis (F and G type). More than one type of virus may produce the same clinical syndrome and one type of virus may cause clinically different diseases.

CULTIVATION

• Human adenoviruses reproduce only in cell cultures of human origin (HeLa, HEP-2, etc.). CPE may take several days to develop and consist of cell rounding and aggregation into grape like clusters.

• Oncogenic human adenoviruses (types 12, 18, 31) cause sarcomas when injected into newborn hamsters. However, there is no evidence at all relating adenoviruses to natural malignancy in humans or animals.

CAUSED DISEASES

Adenovirus infections are common worldwide mostly in children. Many infections are asymptomatic. The virus can persist in the host for many months. Adenoviruses cause ARVI, eye infections, and less often of the intestine and urinary tract.

❖ Most infections with adenovirus result in infections of the URT. Adenovirus infections often show up as **tonsillitis** (which may look exactly like strep throat) and **pharyngitis**. A combination of conjunctivitis and tonsilitis is particularly common with adenovirus infections (acute **pharyngoconjunctival fever**). Some children (especially small ones) can develop adenovirus **bronchiolitis** or **pneumonia**, both of which can be severe. In babies, adenoviruses can also cause coughing fits that look almost exactly like whooping cough (**pertussis-like syndrome**). **Acute Respiratory Disease** (tracheobronchitis, pneumonia, fever) is characteristic during epidemics among military recruits.

❖ Eye infections: **epidemic keratoconjunctivitis** (EKC), **acute follicular conjunctivitis** (nonpurulent inflammation of the conjunctiva with enlargement of lymphoid follicles; clinically similar with chlamydial conjunctivitis).

❖ Adenoviruses can also cause viral **meningitis** or **encephalitis**.

❖ Rarely, adenovirus can cause **hemorrhagic cystitis**.

❖ Adenoviruses, enteric types 40 and 41 (group F) can also cause **gastroenteritis**. Diarrhea tends to last longer than with other viral gastroenteritides (especially in children < 4 years old).

EPIDEMIOLOGY

• Source of infection is anthroponosis for the most of the infections.

• Mechanisms of transmission are air borne or water-borne (swimming). Adenoviruses are often transmitted by expectorate, but can also be transmitted by contact with an infected person, or by virus particles left on objects such as towels and instruments. Some people with adenovirus gastroenteritis may shed the virus in their stools for months after getting over the symptoms.

IMMUNITY, PREVENTION AND TREATMENT

• Immunity is relatively long-lasting but type-specific.

• Treatment is symptomatic. No vaccine is available for general use.

• Nonspecific prophylaxis is achieved by maintaining of asepsis, sterilization of the medical instruments, chlorination of swimming pools, etc. As with many other illnesses, good hand washing is one way to inhibit the spread of

adenoviruses from one person to another. Avoiding use ophthalmological instruments in multiple persons is recommended.

Lecture 18. VIRUSES ARE CAUSATIVE AGENTS OF ACUTE INTESTINAL VIRAL INFECTIONS. HEPATOTROPIC VIRUSES

Viral gastroenteritis (**a**cute **i**ntestinal **v**iral **i**nfection — AIVI) is an inflammation of the stomach and intestine caused by one of any number of viruses (also known as the *stomach flu*). People at higher risk are: children under age five, older adults, especially if they live in nursing homes, children and adults with weakened immune system.

Symptoms often appear within 4 to 48 hours after contact with the virus. Common symptoms include: *abdominal pain*, *diarrhea*, *nausea* and *vomiting*. Other symptoms: chills, clammy skin, sweating, fever, joint stiffness or muscle pain, poor feeding and weight loss. The main complication of viral gastroenteritis is *dehydration*, which can be quite severe in babies and young children.

Table 30 — Characteristics of the causative agents of AIVI

Family	Common viruses as agents of AIVI	Caused infection and principal syndromes
Adenoviridae	Adenoviruses types 40 and 41	*Adenoviral gastroenteritis* (diarrhea tends to last longer than with other viral gastroenteritides, especially in children < 4 years)
Reoviridae	Rotaviruses	*Rotaviral gastroenteritis* (most severe gastroenteritis in children)
Picornaviridae	Enteroviruses and Parechoviruses	*Enteroviral gastroenteritis* ("abdominal discomfort" during many enteroviral infections, including abortive form of poliomyelitis), *"summer diarrhea", infantile diarrhea*
Caliciviridae	Noroviruses Sapoviruses	*Noroviral gastroenteritis* *Sapoviral gastroenteritis*
Astroviridae	Astroviruses	*Astroviral gastroenteritis*
Coronaviridae	Toroviruses	*Toroviral gastroenteritis* (watery diarrhea in infants)
Herpesviridae	Cytomegalovirus (CMV)	*Colitis* in the immunocompromised persons

The main focus of treatment is to prevent dehydration by use of oral rehydration solutions (OHS). In severe cases, hospitalization and intravenous fluids are necessary. Most cases of viral gastroenteritis resolve over time without specific treatment. Antibiotics are not effective against viral infections. Over-the-counter medicines such as loperamide (Imodium) and bismuth subsalicylate (*Pepto-Bismol*) can help relieve symptoms in adults.

People may be infected with viruses by:

✓ touching contaminated surfaces and then touching their mouth; especially if they do not wash their hands thoroughly after using the bathroom;

✓ sharing food, drink, or eating utensils with infected people;

✓ eating foods that are contaminated with the virus, such as oysters from contaminated waters;

✓ swallowing or inhalation of airborne particles that contain viruses ("aerosolized" vomit particles).

Infected people who do not have symptoms can still transmit viruses. Viruses may be present in the stool up to 2 weeks after a person recovers from gastroenteritis. Outbreaks of viral gastroenteritis can occur in households, child-care settings, schools, nursing homes, cruise ships, camps, restaurants and other places, where people gather in groups.

REOVIRUSES

CLASSIFICATION

Group: Group III (ds RNA)
Order: Unassigned
Family: Reoviridae

Subfamily: Sedoreovirinae	Subfamily: Spinareovirinae
Genus: Rotavirus	Genus: Coltivirus
Species: **Rotavirus A-E**	Species: **Colorado tick fever virus**
Genus: Orbivirus	Genus: Orthoreovirus
Genus: Seadornavirus	Species: mammalian **orthoreovirus**
Species: **Banna virus**	

There are five groups (species) of this virus, referred to as A, B, C, D, E. **Rotavirus A**, the most common species, causes more than 90 % of infections in humans. All five species can cause diseases in animals.

MORPHOLOGY

• The name "*Reo*-" is derived from **r**espiratory **e**nteric **o**rphan viruses. The term "*orphan virus*" refers to the fact that some of these viruses have been observed not associated with any known disease. Even though viruses in the Reoviridae family have more recently been identified with various diseases, the original name is still used.

• Reoviruses are **simple** and have a **cubic capsid** composed of an outer and inner protein shell ("double shelled capsid"). The **dsRNA** genome of viruses contains 10–12 segments.

• The electron microscopic appearance of rotavirus (RV) shows a 60–80 nm wheel with radiating spokes (from Latin, *rota* = wheel). Complete or "*double shelled*" viruses and incomplete or "*single shelled*" viruses are seen. The rotavirus genome contains ds RNA in 11 segments.

• Rotaviruses are stable in the environment for many months and are relative resistant to hand washing and lipid solvents.

ANTIGENIC STRUCTURE

• There are 6 **v**iral **p**roteins (VP) that form the virion. These **structural proteins** are called VP1, VP2, VP3, VP4, VP6 and VP7. In addition to the VPs, there are 6 **nonstructural proteins** (NSP) that are only produced in cells infected by rotavirus. These are called NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6. VP7 and VP4 are outer structural proteins are. VP4 is the viral hemagglutinin and forms spikes from the surface. VP 1, 2, 3 and 6 are inner core structural proteins are. VP6 is an important antigenic determinant.

• There are many serotypes of rotaviruses. The glycoprotein VP7 defines the **G-serotypes** and the protease-sensitive protein VP4 defines **P-serotypes**. Because the two genes that determine G-types and P-types can be passed on separately to progeny viruses, different combinations (serotypes) are found.

CULTIVATION

Cultivation of animal and human RVs in cells requires proteolytic activation of the viral attachment protein using **trypsin**. Continuous cell lines, such as rhesus monkey kidney cells, as well as primary monkey kidney cells, are routinely used for the growth and characterization of RVs. Isolation and cultivation of human RVs from clinical fecal specimens is difficult and adaptation to growth in vitro requires multiple rounds of passage in primary cells.

CAUSED DISEASES

❖ **Rotavirus gastroenteritis** is a mild to severe disease characterised by *vomiting*, *watery diarrhea* and *low-grade fever*. The period of illness is acute. Symptoms often start with vomiting followed by profuse diarrhea. Dehydration is more common in rotavirus infection than in most of those caused by bacterial pathogens, and is the most common cause of death related to rotavirus infection. Symptoms of dehydration include dizziness while standing, decrease in urination and dry mouth or throat. Group A infections are most common. Group B has been associated with outbreaks in adults in China. Group C is responsible for sporadic cases of diarrhea in infants around the world.

Mechanism of the rotavirus diarrhea: malabsorption occurs because of the destruction of enterocytes. The toxic rotavirus protein **NSP4** induces Ca^{2+}-dependent chloride secretion, disrupts reabsorption of water, apparently reduces activity of brush-border membrane *disaccharidases*, and possibly activates the Ca^{2+}-dependent secretory reflexes of the enteric nervous system. Healthy enterocytes secrete lactase into the small intestine; milk intolerance due to lactase deficiency is a symptom of rotavirus infection, which can persist for weeks.

Rotaviruses infect children at a young age. Young infants may be protected due to transplacental transfer of maternal Ab. Older infants and young children (4 months-2 years) tend to be more symptomatic with *diarrhea*. Asymptomatic infections are common, especially in adults. Many cases and outbreaks are nosocomial. It was recently discovered that RVs also play a role in infections of elderly and immunosuppressed, ex.: bone marrow transplant patients.

❖ Mammalian **orthoreovirus** does not really cause a significant disease in humans. Even though the virus is fairly common, the infection produced is either asymptomatic or causes a mild disease which is self-limiting in GIT and RT for children and infants. Symptoms of ARVI are similar to common cold, such as a low-grade fever and pharyngitis.

EPIDEMIOLOGY

- Source of infection is anthroponosis (for rotaviruses).
- Mechanism of rotavirus transmission is fecalo-oral, via contact with contaminated hands, surfaces and objects and possibly by the respiratory route ("aerosolized" vomit particles). Viral diarrhea is highly contagious.

IMMUNITY, PREVENTION AND TREATMENT

- Initial infection does not lead to permanent immunity and reinfection can occur at any age. However, subsequent infections are usually less severe than the primary infection. Due to immunity acquired in childhood, most adults are not susceptible to rotavirus.
- Treatment is just supportive care with rehydration (oral/intravenous). Antiviral agents not known to be effective. Good hand washing technique is important. In addition, surfaces, toilets and toys should be disinfected. Adequate chlorination of water can prevent spread in the community.
- *Rotarix* and *Rotateq* vaccines are available. Rotarix is a live attenuated monovalent rotavirus serotype G1. RotaTeq is a live, oral pentavalent vaccine that contains five rotavirus strains produced by reassortment.

ASTROVIRUSES

CLASSIFICATION

Group: Group IV (+ssRNA)
Family: Astroviridae
Genus: Mamastrovirus
Species: Bovine, Feline, Ovine, Porcine, **Human astroviruses**
Genus Aviastrovirus (birds astroviruses)

MORPHOLOGY

Astroviruses are **simple**, small 28–35 nm, **cubic viruses** that have a characteristic five- or sixpointed *star-like* surface structure when viewed by electron microscopy. Astrovirus has a **nonsegmented, single stranded, positive sense RNA** genome.

ANTIGENIC STRUCTURE

The astroviruses are further classified within each species into serotypes.

CULTIVATION

Astroviruses can be cultivated in the cell cultures.

CAUSED DISEASES

Human astroviruses is important cause of acute **gastroenteritis** in young

children worldwide. The main symptoms are *diarrhea*, followed by *nausea, vomiting, fever, malaise* and *abdominal pain*. Astrovirus infection is not usually a severe situation and only in some rare cases leads to dehydration.

EPIDEMIOLOGY

- Source of infection is anthroponosis.
- Mechanism of transmission is fecalo-oral.

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is nonlonglasting, reinfections are common.
- There is no vaccine is available for common use.
- Prevention is only nonspecific like for other AIVI.

CALICIVIRUSES

CLASSIFICATION

Group: Group IV (+ssRNA)
Family: Caliciviridae
Genus: Norovirus → Species: **Norwalk virus**
Genus: Sapovirus → Species: **Sapporo virus**

MORPHOLOGY

Caliciviruses are **simple**, small 27–40 nm, **cubic** viruses with **nonsegmented, single stranded, positive sense RNA** genome. The name *calicivirus* is derived from the Greek word *calyx* meaning "cup". This name is appropriate as many strains have visible cup-shaped depressions when viewed by electron microscopy.

ANTIGENIC STRUCTURE

The caliciviruses are further classified within each species into serotypes.

CULTIVATION

Caliciviruses are not being cultivated in culture until recently.

CAUSED DISEASES

Calicivirus infections commonly cause acute **gastroenteritis**. Symptoms can include vomiting and diarrhea. Most calicivirus infections do not call for medical attention, but those who are immunocompromised may need to be hospitalized for rehydration therapy.

Norovirus is the most common cause of viral gastroenteritis in humans. It affects people of all ages. Norovirus infection is characterized by nausea, forceful vomiting, watery diarrhea, abdominal pain, and in some cases, loss of taste. General lethargy, weakness, muscle aches, headache, and low-grade fever may occur. The disease is usually self-limiting, and severe illness is rare. Common name of the illness caused by noroviruses still in use includes "*winter vomiting disease*" (minor epidemics during the winter months). It is also known as "*stomach flu*", but this actually is a broad name that refers to gastric inflammation caused by a range of viruses.

Unlike noroviruses, Sapporo viruses generally cause mild gastroenteritis in

young children.

EPIDEMIOLOGY

- Source of infection is anthroponosis.
- Mechanisms of transmission are fecalo-oral, person-to-person contact and via aerosolization of the virus and subsequent contamination of surfaces. Outbreaks of norovirus infection often occur in communities, such as long-term care facilities, overnight camps, hospitals, schools, prisons, dormitories and cruise ships.
- The norovirus can survive for long periods outside a human host depending on the surface and temperature conditions: can stay for weeks on hard surfaces, and up to 12 days on contaminated fabrics, and it can survive for months, maybe even years in contaminated still water.

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is nonlonglasting, reinfections are common.
- There is no vaccine is available for common use.
- Prevention is only nonspecific like for other AIVI.

PICORNAVIRUSES

CLASSIFICATION

Group: Group IV (+ssRNA)
Order: Picornavirales
Family: Picornaviridae
1) Genus: Cardiovirus → human **cardioviruses**
2) Genus: Aphthovirus
→ Species: **foot-and-mouth disease virus** (FMDV)
3) Genus: Hepatovirus → Species: **hepatitis A virus** (HAV)
4) Genus: Parechovirus → Species: human **parechoviruses** (HPeV)
5) Genus: *Enterovirus*:
Species: human **enteroviruses** A, B, C, D → subtypes **Coxsackie A** and **B** viruses, **echoviruses** (ECHO), **polioviruses**, other **enteroviruses**.
Species: human **rhinoviruses** A, B, C (HRV)

MORPHOLOGY

Picornaviruses are **simple, positive-stranded RNA** viruses with a **cubic capsid** composed of four viral proteins (VP1-VP4). The name is derived from *pico*, meaning *small* (20–30 nm). VP4 is bound to viral RNA.

ANTIGENIC STRUCTURE

Serologic studies have distinguished 71 human enterovirus serotypes on the basis of antibody neutralization tests. On the basis of their pathogenesis the enteroviruses were originally classified into four subtypes (groups): **polioviruses** (3 serotypes), **Coxsackie A viruses** (24 serotypes), **Coxsackie B viruses** (6 serotypes), and **echoviruses/ECHO** (34 serotypes). Enteroviruses are isolated more recently are named with a system of consecutive numbers: **EV68, EV69, EV70**, and **EV71**.

➢ Type 1 of poliovirus is the most common and causes most epidemics. Type 2 usually causes endemic infections. Type 3 also causes epidemics but less common.

➢ There are more than 110 serotypes of rhinoviruses, formerly classified as **M** biotypes (culturable in rhesus monkey kidney and human cells) and **H** biotypes (growing only in cultures of human cells).

Table 31 — Antigenic classification of some picornaviruses

Genus	Species	Subtypes and serotypes	Examples
Enterovirus	*Enteroviruses A-D*		
	Enterovirus A	▪Coxsackie A viruses (CV-A) ▪Enteroviruses (EV-A)	CV-A2 CV-A8 EV-A71
	Enterovirus B	▪Coxsackie A9 virus ▪Coxsackie B viruses (CV-B) ▪Enteroviruses (EV-B) ▪Echoviruses (E)	CV-A9 CV-B4, CV-B5 EV-B88 E-12, E-33
	Enterovirus C	▪Polioviruses (PV) ▪Coxsackie A viruses (CV-A) ▪Enteroviruses (EV-C)	PV-1, PV-2, PV-3 CV-A1 EV-C117
	Enterovirus D	▪Enteroviruses (EV-D)	EV-D68
	Rhinoviruses A-C		
	Rhinovirus A	74 human serotypes	HRV A1, A2, A7
	Rhinovirus B	25 human and animal serotypes	HRV B3, B4, B70
	Rhinovirus C	6 human serotypes	HRV C1-C5
Parechovirus	*Parechovirus*	6 serotypes	HPeV1 (formerly E-22), HPeV2 (formerly E-23) and HPeV3,4,5,6
Aphthovirus	*Foot-and-mouth disease virus*	7 serotypes	O, A, C, SAT 1, SAT 2, SAT 3 (**S**outhern **A**frican **T**erritories) and Asia 1

CULTIVATION

• All Enteroviruses are **cytopathic (cytopathogenic) viruses** which can produce CPE in the cell cultures (*degeneration of cells*); also for their indication phenomenon of *plague formation* can be used.

• **Coxsackie** viruses were called so as the patients came from the village of Coxsackie in New York. They are classified into groups A and B based on the pathological changes produced in *sucking mice*. Coxsackie A viruses produce a general myositis and flaccid paralysis leading to death within a week. Coxsackie B viruses produce a focal myositis, spastic paralysis, necrosis of the brown fat and, often, pancreatitis, hepatitis, myocarditis and encephalitis. All Coxsackie viruses can be readily cultivated in *cell cultures* producing CPE.

• **ECHO** viruses (echoviruses) is meaning "**e**nteric **c**ytopathic **h**uman **o**r-

phan" viruses as they could not be associated with any particular clinical disease upon time of their discovery. These viruses are not pathogenic for laboratory animals and can be cultivated only in *cell cultures* producing CPE.

• **Polioviruses** are readily cultivated in the *cell cultures* producing characteristic CPE. Experimentally, monkeys may be infected by intracerebral and intraspinal inoculation.

• **Rhinoviruses** can be cultivated in the *cell cultures* of human and monkey origin (CPE is rounding and enlargement of the infected cells).

CAUSED DISEASES

❖ Human **parechoviruses** cause mild, gastrointestinal ("summer diarrhea") or respiratory illness, but have been implicated in cases of myocarditis and encephalitis.

❖ Clinical forms of **enteroviral infections** caused by **nonpolio viruses** (Coxsackie A and B, echoviruses, other enteroviruses):

✓ *Polio-like syndrome* found in children with flaccid paralysis.

✓ *Undifferentiated febrile illness* is the most common presentation of enterovirus infection. Other than fever, symptoms include muscle pain, sore throat, gastrointestinal discomfort and headache.

✓ Minor respiratory infections resembling *common col.*

✓ *Aseptic meningitis* may be caused by most group A and all group B viruses (in children).

✓ *Bornholm disease* or *epidemic pleurodynia* is characterized by severe paroxysmal pain in the chest and abdomen, along with fever, sometimes nausea, headache and emesis.

✓ *Pericarditis* and/or *myocarditis*.

✓ *Encephalomyocarditis* in the newborns associated with high mortality may be caused by group B viruses.

✓ Acute *hemorrhagic conjunctivitis*.

✓ *Herpangina* is caused by Coxsackie A virus (vesicular pharyngitis).

✓ *"Hand, foot and mouth disease"* is a childhood illness most commonly caused by infection by Coxsackie A virus or EV-71; a common childhood illness with fever and painful blisters in the mouth (herpangina), on the palms and fingers of the hand, or on the soles of the feet. Adults can also be affected.

✓ *Birth defects* (congenital infection) may also be present in a Coxsackie B infected infant.

✓ The B4 strain of Coxsackie viruses has been discovered to be a possible cause of *Diabetes mellitus type 1*.

✓ Type B viruses have been associated with the condition called *postviral fatigue syndrome*.

✓ Other clinical forms: *gastroenteritis* in adults, *infantile diarrhea*, *exanthem*, *hepatitis*, *pancreatitis*, etc.

✓ Enterovirus types 68 and 69 cause common cold; EV-70 causes acute

hemorrhagic conjunctivitis; EV-71 can cause *meningitis, encephalitis* and outbreaks of *hand-foot-mouth disease* with or without *encephalitis.*

❖ **Poliomyelitis** (pathogenesis and clinical forms).

Poliovirus is an enterovirus. Virus is shed in the feces of infected individuals. The virus multiplies initially in the *epithelial cells* of GIT and the *lymphatic tissues* (tonsils and Peyer's patches). In 95 % of cases only a *primary, transient viremia* occurs, and the poliovirus infection is ***asymptomatic (inapparent)*** which causes only seroconversion. In about 5 % of cases, the virus spreads and replicates in other sites such as *brown fat, reticuloendothelial tissue* and *muscle*. The sustained viral replication causes *secondary viremia* and leads to the development of minor symptoms such as fever, headache and sore throat (***abortive infection*** or *mild illness*). If the infection progresses, the minor illness is fallowed 3–4 days later by the major illness. The fever comes again along with headache, stiff neck and other symptoms of *aseptic meningitis*. This marks the stage of viral replication in CNS. Sometimes the disease does not progress beyond this stage (***nonparalytic poliomyelitis***). In those proceeding to paralytic infection, flaccid paralysis develops. ***Paralytic poliomyelitis*** occurs in less than 1 % of poliovirus infections. Paralytic disease occurs when the virus replicates in the spinal cord, brain, or motor cortex, resulting in the selective destruction of motor neurons leading to temporary or permanent paralysis (can be spinal, bulbar or bulbospinal polio). The mechanisms by which poliovirus enters the CNS are poorly understood (virions pass directly from the blood into CNS by crossing the blood–brain barrier; virions are transported from muscle tissue to the spinal cord through nerve pathways via retrograde axonal transport or virus is imported into the CNS via infected monocytes or macrophages).

Recovery takes place next 4–8 weeks and is usually complete after 6 months, leaving behind varying degrees of *residual paralysis*.

❖ Humans can be infected with **foot-and-mouth disease** (FMD) through contact with infected animals, but this is extremely rare. Symptoms of FMD in humans include malaise, fever, vomiting, red ulcerative lesions of the oral tissues, and sometimes vesicular lesions (blisters) of the skin.

❖ Human **cardioviruses** have been associated with gastroenteritis, ARVI and nonpolio associated acute flaccid paralysis in North America, Europe and South Asia.

❖ **Rhinoviral infection** includes sore throat, runny nose, nasal congestion, sneezing and cough (rhinoviruses preferentially replicate at 32 °C as opposed to 37 °C, hence infect URT). Pathogenesis: entry of human rhinoviruses in URT (mouth and nose) → virus binding to *ICAM-1* (Inter-Cellular Adhesion Molecule, CD54) on respiratory epithelial cells → replication of virus → infected cells release "distress signals" (chemokines/cytokines) → inflammatory leukocytes, preferentially granulocytes and monocytes, are found to migrate to the site of HRV infection → cell lysis. It is now considered that common cold symptoms result from an inflammatory "*cytokine disease*". It is now well acknowl-

edged that HRVs are involved in the exacerbations of asthma, cystic fibrosis, chronic obstructive pulmonary disease, pneumonia, sinusitis, otitis media and wheezing of infants. The recently discovered HRV-C appears to give rise to more severe respiratory tract illness especially in pediatric patients. HRV-C infections, in addition to symptoms of the common cold, cause pharyngitis, croup, otitis media, bronchiolitis, or pneumonia.

EPIDEMIOLOGY

- Source of enteroviral and rhinoviral infections is anthroponosis.
- Mechanisms of enteroviral transmission are fecalo-oral; person-to-person contact (via the contaminated hands of hospital personnel) and via aerosolization of the viruses and subsequent contamination of surfaces (indirect contact). Contaminated swimming pools can also transmit the virus.

Main causes of infection are from overcrowded conditions such as the poor districts of a city and poor hygiene.

- Mechanisms of transmission of poliomyelitis are fecalo-oral and inhalation of respiratory secretions (possible in close contact in early stage of the disease).
- There are two modes of transmission of rhinoviruses: via aerosols of respiratory droplets and from contaminated surfaces, including direct person-to-person contact.
- *Picornaviruses* are highly resistant viruses. Enteroviruses replicate at 37 °C, whereas rhinoviruses grow better at 33 °C, as this is the lower temperature of the nose. Enteroviruses are stable under acid conditions and thus they are able to survive exposure to gastric acid. In contrast, rhinoviruses are acid-labile (destroyed by low pH) and that is the reason why rhinovirus infections are restricted to RT.

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is type specific (for enteroviruses).
- Immunity after poliomyelitis is type-specific, however second attacks within the same individual are extremely rare.
- Vaccines against poliomyelitis are **OPV** (**o**ral attenuated **p**olio **v**accine or *Sabin* vaccine) and **IPV** (**i**nactivated parenteral **p**olio **v**accine or *Salk* vaccine). OPV is not safe in immunodeficient subjects (can induces **v**accine-**a**ssociated **p**aralytic **p**oliomyelitis — **VAPP**). In immune individuals, sIgA antibodies against poliovirus are present in the tonsils and GIT ("local immunity" created with OPV) and are able to block poliovirus replication; IgG and IgM antibodies against poliovirus ("system immunity" created by IPV/OPV) can prevent the spread of the virus to motor neurons of CNS. Immunity fallowing IPV may need to be maintained by booster doses periodically, while immunity following OPV resembles natural active immunity in being more lasting.
- Treatment for enteroviral infection is mainly supportive.
- Control of picornavirus diseases depends largely on mass education of the public on the mode of virus transmission, stressing the importance of good personal hygiene and on provision of a good sewage disposal system and uncontaminated water supply.

• Human rhinovirus is extremely contagious during the cold months of each year. The virus can live up to 3 hours outside human contact. It is important to wash hands on a regular basis and avoid touching the mouth or nose.

HEPATOTROPHIC VIRUSES

The term "*viral hepatitis*" refers to a primary infection of the liver by hepatitis viruses (A, B, C, D, E, G, TTV, etc.). Hepatitis viruses are taxonomically unrelated. Except for type B which is DNA virus all other are RNA viruses (recently discovered TTV is also ssDNA virus). The features common for all of them are their **hepatotropism** and ability to cause a similar icteric illness, ranging in severity from mild and inapparent/latent forms to the fulminant fatal forms. Hepatitis may occur incidentally during many other viral infections such as yellow fever, Lassa fever, Ebola fever, herpes infections but these are not included in the category of viral hepatitis.

Table 32 — Differentiation of viral hepatitis viruses

Virus	Family	Genus	Genome	Enve-lope	Mechanism of transmission
HAV	Picornaviridae	Hepatovirus	+ss RNA	-	Fecalo-oral
HBV	Hepadna-viridae	Orthohepad-navirus	dsDNA (defect)	+	Parenteral
HCV	Flaviviridae	Hepacivirus	+ssRNA	+	Parenteral
HDV	*Unclassified*	Deltavirus	-ssRNA	-	Parenteral
HEV	Hepeviridae	Hepevirus	+ssRNA	-	Fecalo-oral
HGV	Flaviviridae	Pegivirus	+ssRNA	+	Parenteral
TTV*	Anelloviridae	Alphator-quevirus	ss DNA	-	Parenteral

*TTV — transfusion-transmitted virus

By epidemiological and clinical criteria, two types of viral hepatitis had been recognized — enteral and parenteral. **Enteral hepatitis** consist of hepatitis A and E (they occur sporadically or as epidemics, affecting mainly children and young adults, represented as acute forms, and transmitted by the fecalo-oral mechanism). **Parenteral hepatitis** (blood-borne or serum hepatitis) consist of mainly hepatitis B, C and D. They affect all ages, represented as acute and persistent (chronic) clinical forms, can lead to development of liver cirrhosis and cancer, and transmitted by parenteral (blood-borne) mechanism (sexual, transplacental, injections, blood transfusion and other medical and nonmedical, such as tattoo, piercing, manicure, manipulations).

Differentiation of hepatitis is based on their **serological markers** (antigens and antibodies which are used in laboratory diagnosis).

HEPATITIS A VIRUS

CLASSIFICATION

Group: Group IV (+ssRNA)
Order: Picornavirales
Family: Picornaviridae
Genus: Hepatovirus

Species: **Hepatitis A virus** (HAV)

MORPHOLOGY

HAV is small **simple +ssRNA** virus with **cubic** symmetry of **capsid**.

ANTIGENIC STRUCTURE

There is only one serotype of the virus.

CULTIVATION

The virus grows poorly in primate cellular cultures and loses virulence by in vitro passages. It is the only human hepatitis virus which can be cultivated in vitro (but cultivation is prolonged and CPE is not expressed). Chimpanzees can be infected experimentally.

CAUSED DISEASES

Hepatitis A (formerly known as *infectious hepatitis*) is an acute infectious disease of the liver caused by HAV. The time between infection and symptoms, in those who develop them, is between 2–6 weeks. The most of infections are *subclinical*. The clinical disease consists of two stages: prodromal or preicteric and icteric. Prodromal symptoms are fever, malaise, nausea, vomiting, diarrhea, abdominal pain and liver tenderness. These symptoms usually subside with the onset of ***jaundice***. The disease is milder in children, in whom many infections may be anicteric. Mortality is low (<1 %), with most of the death occurring in adults (fatal fulminant hepatitis).

Pathogenesis of hepatitis A: following ingestion, HAV multiplies in the intestinal epithelium → enters the bloodstream (brief viremia during preicteric stage) → blood carries the virus to the ***liver***, where it multiplies within hepatocytes and Kupffer cells (liver macrophages). There is no apparent virus-mediated cytotoxicity and liver pathology is likely immune-mediated. Virions are secreted into the bile and released in stool. HAV is excreted in large quantities during the late incubation period and prodromal stage of illness prior to appearance of symptoms or anti-HAV IgM antibodies in the blood. Once jaundice develops, HAV and its antigens (HAV-Ag) are rarely detectable in the feces. Chronic carriers are not seen.

EPIDEMIOLOGY

- Source of infection is anthroponosis.
- Mechanism of transmission is **fecalo-oral** (parenteral transmission is very rare because of chronic viremia does not occur). Hepatitis A occurs sporadically or as outbreaks, which may be caused by contaminated food, water, shellfish or milk. Domestic or institutional spread of infection among children is common. Overcrowding and poor sanitation favor its spread.

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is long-lasting. There is no cross immunity between HAV and any other hepatitis viruses.

• There are two types of vaccines: one containing inactivated hepatitis A virus, and another containing a live but attenuated virus.

• Specific passive prophylaxis by pooled normal human Ig before exposure or in early incubation period can prevent or attenuate clinical illness.

• Treatment is symptomatic (no specific antiviral drug is available).

• In developing countries, and in regions with poor hygiene standards, the rates of infection with this virus are high and the illness is usually contracted in early childhood.

HEPATITIS E VIRUS

CLASSIFICATION

Group: Group IV (+ssRNA)
Family: Hepeviridae
Genus: Hepevirus
Species: **Hepatitis E virus** (HEV)

MORPHOLOGY

HEV is small **simple +ssRNA** virus with **cubic** symmetry of **capsid**.

ANTIGENIC STRUCTURE

There is only one serotype of the virus, but it consists of four genotypes. Genotype 1 has been isolated from tropical and several subtropical countries in Asia and Africa. Genotype 2 has been isolated from Mexico, Nigeria, and Chad. Genotype 3 has been isolated almost worldwide including Asia, Europe, North and South America. Genotype 4 appears to be limited exclusively to Asia.

Genotypes 1 and 2 are restricted to humans and often associated with large outbreaks and epidemics in developing countries with poor sanitation conditions. Genotypes 3 and 4 infect humans, pigs and other animal species and have been responsible for sporadic cases of hepatitis E in both developing and industrialized countries.

CULTIVATION

Cultivation of HEV in the cell cultures is very difficult.

CAUSED DISEASES

Hepatitis E (non-A-non-B hepatitis, epidemic NANB hepatitis) is often *acute* and self-limiting infection (in that it usually goes away by itself and the patient recovers) with low mortality rates (2 %). Clinically, Hepatitis E is comparable to hepatitis A, but in pregnant women the disease is more often severe and is associated with fatal fulminant hepatitis (pregnant women, especially those in the third trimester have mortality rate ~20 %). The incubation period of hepatitis E varies from 3 to 8 weeks. Symptoms may include jaundice, fatigue and nausea.

While usually an acute disease, in immunocompromised subjects (particularly in organ transplanted patients) hepatitis E may cause a chronic infection.

EPIDEMIOLOGY

• Source of infection is zooanthroponosis. Domestic animals have been reported as a reservoir for the hepatitis E virus (revealed infection rates exceeding

95 % among domestic pigs; rabbit hepatitis E virus has also been described).

• Mechanisms of transmission are fecalo-oral, principally via contaminated water; also foodborne transmission from ingestion of products derived from infected animals.

• HEV causes acute sporadic and epidemic viral hepatitis. It is prevalent in most developing countries, and common in any country with a hot climate (Southeast Asia, northern and central Africa, India, and Central America).

• Outbreaks of epidemic hepatitis E most commonly occur after heavy rainfalls and monsoons because of their disruption of water supplies.

• Symptomatic infection is most common in young adults aged 15–40 years.

IMMUNITY, PREVENTION AND TREATMENT

• Immunity is long-lasting.

• Preventative vaccine (HEV 239) is approved for use in China; although it is not yet available globally.

• The risk of infection and transmission can be reduced by maintaining quality standards for public water supplies.

HEPATITIS B VIRUS

CLASSIFICATION

Group: Group VII (dsDNA-RT)
Order: Unassigned
Family: Hepadnaviridae
Genus: Orthohepadnavirus
Species: **Hepatitis B virus** (HBV)

MORPHOLOGY

• HBV is 42 nm **dsDNA complex** virus with **cubic** symmetry of **capsid**.

• Under the electron microscopy blood from patient with hepatitis B shows 3 types of particles: (a) *spherical particle* 22 nm in diameter; (b) *filamentous* or *tubular* particles with diameter of 22 nm and of varying length → these two particles are antigenically identical and are surface components of HBV (HBsAg – hepatitis B surface antigen) → they are produces in great excess and noninfective; (c) the third type of particle, less in number, is *spherical virions* 42 nm in diameter (also known as *Dane particle*). Only Dane particle is the infective form of HBV.

• Genome of HBV is dsDNA held in circular configuration. One of the strand (+ strand, internal) is incomplete, so that DNA appears partially **ds** and partially **ss** (*defective dsDNA*).

• HBV replicates within hepatocytes. Viral DNA exists in the hepatocytes nucleus in the free extrachromosomal state (*autonomous infection*) or integrated with the cell chromosome (*integrative infection*). Replication resembles that seen in HIV, in that DNA is synthesized from RNA template by **reverse transcription**.

ANTIGENIC STRUCTURE

• **HBsAg** ("*Australian antigen*" because physician Blumberg and described

the presence of antibodies to these antigens in the serum of Australian aborigines). HBsAg is the *most immunogenic* antigen of HBV (used for creation of recombinant vaccine; antiHBs antibodies possess more powerful protective effect). HBsAg is the first marker to appear in blood, even before onset of clinical illness. In typical case (acute hepatitis B), it disappears within 2 months of the start of disease, but if it persists longer (> 6 months) is a predictor of chronic hepatitis B.

• **HBcAg** is core (capsid) antigen. It is not secreted and does not circulate in blood, but expressed on the surface of the infected hepatocytes. Antibodies to HBcAg (antiHBc-IgM and antiHBc-IgG) appear in blood and are useful serological markers of acute/chronic hepatitis B.

• **HBeAg** is a soluble nucleocapsid antigen is also derived from HBcAg gene (HBcAg and HBcAg are coded for by the same gene). HBeAg is posttranslational, truncated form of HBcAg. It is released by the infected hepatocytes into blood and its presence denotes high infectivity of blood (high level of virus replication).

• **HBxAg** is transactivator regulating viral transcription. This antigen can be detected only in the state of primary liver cancer (hepatocellular carcinoma).

➢ Two type of mutation have been studied. One type is severe chronic hepatitis caused by *HBeAg negative mutants*. The second group is called "*escape mutants*" that show mutation in the genes responsible for synthesis of HBsAg; this prevents HBsAg-mutants from being neutralized by antiHBs.

CULTIVATION

HBV does no replicate in any conventional cell culture. But limited production of the virus can be obtained from several cell line transfected with DNA of HBV. HBV proteins have been cloned in bacteria and yeast. The chimpanzee is susceptible to experimental infection and can be used as laboratory model.

CAUSED DISEASES

Hepatitis B is the most widespread and the most important type of viral hepatitis. The incubation is long (1–6 months). Clinically, hepatitis B is similar to hepatitis A but it is more severe and protracted. The onset of the disease is insidious and prodromal stage (fever, malaise) is not prominent. Extrahepatic manifestations (skin rash, arthralgia, polyarthritis, glomerulonephritis) may occur. About 90–95 % patients with acute hepatitis B recover and eliminate virus from the body within ~ 6 months. Mortality is very low (0,5–2 %). But 5–10 % of patients remain *chronically* infected. They may be *asymptomatic carriers* or may progress to *recurrent* or *chronic liver disease* or *cirrhosis*. A few of them may develop *hepatocellular carcinoma* after many years.

The pathogenesis of hepatitis B appears to be *immune mediated* (hepatocytes carry viral Ag and are subjects to cytotoxic T-cells attack or ADCC mediated by NK cells).

In general, three *clinical forms of hepatitis B* are differentiated:

(1)*Healthy (asymptomatic)HBV carriers* (latent infection);

(2)***C****hronic* ***p****ersistent* ***h****epatitis* (**CPH**) without viral reproduction;

(3)***C****hronic* ***a****ggressive* ***h****epatitis* (**CAH**) with viral reproduction and pro-

gressive course of disease (finally can lead to cirrhosis or cancer).

EPIDEMIOLOGY

• Source of infection is anthroponosis (sick persons and especially carriers whose blood contains circulating virus for long periods, even lifelong). **Carrier** is a person with detectable HBsAg in blood for > 6 months. Carriers are of two categories — *highly infectious supercarriers* and *simple carriers*. Supercarrier has high titre HBsAg, along with HBeAg and DNA (also generally elevated transaminases). Simple carrier has low infectivity and low titre HBsAg in blood with negative HBeAg/DNA. Many supercarriers in time become simple carriers.

• Mechanisms of transmission are parenteral, sexual and perinatal. It should be assumed that all bodily fluids from HBV-infected patients may be infectious (blood, saliva, urine, semen, vaginal secretion, synovial fluid, CSF, breast milk).

• Hepatitis B occurs throughout the world. The infection is usually sporadic, though occasional outbreaks have occurred (in hospitals). The prevalence of hepatitis carriers varies widely in relation to the living standards. Regions with high endemicity are equatorial Africa, South East Asia, China. Low endemicity is characterized for developed countries.

• There is no seasonal trend for HBV infection and no high predilection for any age group, although there are definite *high-risk groups* such as parenteral drug abusers, institutionalized persons, health care personnel, multiply transfused patients, organ transplant patients, hemodialysis patients and staff, highly promiscuous persons, and newborn infants born to mothers with hepatitis B. Since mandatory screening of blood donors for HBsAg was instituted, the number of cases of transfusion-associated hepatitis has been dramatically reduced. However, HBsAg screening is not a totally failsafe method as infection has occurred even with HBsAg negative but antiHBc positive blood, which may have had undetectable amounts of virus.

• People have been infected *by improperly sterilized syringes, needles, or scalpels* and even by *tattooing* or *ear piercing*. HBsAg can be detected in saliva, nasopharyngeal washings, semen, menstrual fluid, and vaginal secretions as well as in blood. Transmission from carriers to close contacts by the *oral route* or by *sexual* or other intimate exposure occurs. There is strong evidence of transmission from persons with subclinical cases and carriers of HBsAg to homosexual and heterosexual long-term partners. Subclinical infections are common, and these unrecognized infections represent the principal hazard to hospital personnel.

• Infection by direct contact with open skin lesion (pyoderma, cuts, scratches, etc.) is very common among young children in developing countries, as also through household transmission.

• Perinatal (congenital, vertical) transmission is quite common from carrier mother (if mother is HBeAg positive risk is 60–90 % and 5–15 % if mother is HBeAg negative). True congenital infection (*in utero*, transplacental) is rare. It is believed that perinatal infection occurs mostly during the time of delivery.

Most individuals infected as infants remain carriers for life and some of them may develop liver cancer after many decades.

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is long-lasting (presence of antiHBs, antiHBe, antiHBc-IgG).
- Specific prevention: hepatitis B vaccine is produced by recombination of virus DNA in yeast cells in which genome a plasmid containing the gene of HBsAg has been incorporated (**recombinant vaccine**). Periodic booster shots are recommended for some time to maintain sufficient immune protection.
- Passive immunization with hyperimmune **h**epatitis **B** **i**mmune **g**lobulin (**HBIG**) is used after exposure to infection.
- Treatment is symptomatic treatment. Etiological treatment by recombinant *alpha-IFN* and *Lamivudine* (reverse transcriptase inhibitor) can be done. There is no effective treatment for the carrier state, though spontaneous resolution takes place sometimes.
- Nonspecific prophylaxis consists of avoiding risky practices like promiscuous sex, injectable drug abuse, health education, screening of blood, semen and organ donors, etc.

HEPATITIS D VIRUS

- **Hepatitis D virus** is unclassified virus in its own genus Deltavirus (hepatitis delta virus or **delta agent**).
- HDV is a small, **simple**, spherical virus with a 36 nm diameter. It has an outer coat containing HBsAg. The **cubic** nucleocapsid contains **negative sense, single-stranded, closed circular RNA** and hepatitis D antigen (HDAg).
- HDV is considered to be a **subviral satellite** because it can propagate only in the presence of HBV (in this case the "*helper virus*"). HDV has no independent existence and can survive only as long as HBV infection persists in the host.
- Transmission of HDV can occur either via simultaneous infection with HBV (**coinfection**) or superimposed on chronic hepatitis B/carrier state (**superinfection**). Both infections with HDV results in more severe complications compared to monoinfection with HBV. These complications include a greater liver failure and a rapid progression to liver cirrhosis, with an increased chance of developing liver cancer in patients with chronic hepatitis B (no direct association has been noted between HDV and liver cancer). In combination with hepatitis B virus, hepatitis D has the highest mortality rate among all the hepatitis (20 %).
- Hepatitis D is also parenteral hepatitis which can represent in **acute** or **chronic** clinical forms.
- HDV is distributed worldwide but is more common in the immediate Mediterranean region, sub-Saharan Africa, the Middle East, and northern part of South America.
- The vaccine for hepatitis B protects against hepatitis D.

HEPATITIS C VIRUS

CLASSIFICATION

Group: Group IV (+ssRNA)
Order: Unassigned
Family: Flaviviridae
Genus: Hepacivirus → Species: **Hepatitis C virus** (HCV)

MORPHOLOGY

• HCV is small (55–65 nm in size), **enveloped**, **positive-sense single-stranded RNA** virus with **cubic** symmetry of **capsid**.

• HCV replicates mainly in the *hepatocytes* and also can replicate in *peripheral blood mononuclear cells* → high level of immunological disorders found in chronically infected HCV patients.

• HCV shows considerable genetic and antigenic diversity.

• HCV contributes to liver cancer by modulating pathways that may promote malignant transformation of hepatocytes. At least NS3 and NS5 play role in several potentially oncogenic pathways (promotion of cell growth, inhibition of apoptosis, induction of oxidative stress, etc.).

ANTIGENIC STRUCTURE

• HCV mutates rapidly due to a high error rate in RNA polymerase gene → production of many variants of the virus (they are considered as **quasispecies** rather than a conventional virus species).

• HCV species is classified into seven **genotypes** (1–7) with several **subtypes** within each genotype (ex.: 1b). Subtypes are further broken down into quasispecies based on their genetic diversity. Genotypes 1a and 1b are found worldwide and cause 60 % of all cases. Genotype is clinically important in determining potential response to IFN-based therapy. For example, duration of therapy for genotypes 1 and 4 is 48 weeks, whereas for genotypes 2 and 3 is 24 weeks. Infection with one genotype does not confer immunity against others and coinfection with two strains is possible. In most of these cases, one of the strains removes the other from the host in a short time. This finding opens the door to replace strains *nonresponsive* to medication with others easier to treat.

• **E**nvelope glycoproteins are E1 and E2 (E1-Ag serves as fusion gp and E2-Ag acts as the receptor binding protein).

• **N**on**s**tructural proteins (**NS**) are NS2, NS3, NS4A, NS4B, NS5A and NS5B.

CULTIVATION

HCV cannot be cultivated in cell cultures.

CAUSED DISEASES

Hepatitis C infection causes *acute* symptoms in 15 % of cases. Symptoms are generally mild or anicteric including a decreased appetite, fatigue, nausea, muscle or joint pains and weight loss (over jaundice is seen in 5 % of patients only). The infection resolves spontaneously in about >10 % of cases. About 80–85 % of those exposed to the virus develop a *chronic infection*. This is defined as the presence of

detectable viral replication for at least 6 months. Chronic infection after several years may cause cirrhosis or liver cancer.

The most common problem due to hepatitis C but not involving the liver is mixed *cryoglobulinemia* (inflammation of small blood vessels). Hepatitis C is also associated with some autoimmune disorders (thrombocytopenia, diabetes mellitus, autoimmune thyroiditis, etc.).

EPIDEMIOLOGY

- Source of infection is anthroponosis (especially asymptomatic carries).
- Mechanism of transmission is parenteral (very low risk of sexual or vertical transmission). Groups of risk are injecting drug users, people who are transfused blood, transplant patients and sometimes patients on hemodialysis. Common setting for transmission of HCV is also nosocomial transmission, when practices of hygiene and sterilization are not correctly followed in the clinic.
- WHO estimated in 1997 that about 3 % of the world population has been infected (however, in Africa and Asia prevalence rate > 10 %).

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is genotype-specific.
- Only general prophylaxis, such as blood screening, is possible.
- No specific active or passive immunization is available.
- Prolonged treatment with alpha-IFN (*Peginterferon*), either alone or in combination with antiviral agents like *Ribavirin* has been reported to be useful in some cases.

HEPATITIS G VIRUS

- **GB virus C** (GBV-C), formerly known as hepatitis G virus (HGV) is known to infect humans, but is not known to cause human disease.
- GB Virus C is named after the surgeon, G. Barker, who fell ill in 1966 with a non-A-non-B (NANB) hepatitis which at the time was thought to have been caused by a new, infectious hepatic virus.
- GBV-C is a member of the Flaviviridae family and is related to HCV, but replicates primarily in lymphocytes and poorly in hepatocytes.
- GBV-C infection has been found worldwide. High prevalence is observed among subjects with the risk of parenteral exposures (intravenous drug users).

Lecture 19. ECOLOGICAL GROUP OF ARBOVIRUSES AND VIRUSES WITH NATURAL FOCI

➢ **Arboviruses** (**ar**thropod-**bo**rne viruses) are viruses of vertebrates biologically transmitted by ***hematophagous insect vectors***. Inclusion in this group of viruses is based on ecological and epidemiological considerations and hence it contains members that dissimilar in other properties (taxonomy, morphology, cultivation). The name "*arbovirus*" is ecological group of taxonomically unrelated viruses. Arboviruses have been placed in Toga-, Flavi-, Bunyaviridae and other families (table 33). Within each family they are classified into genera, species and antigenic groups.

Table 33 — Taxonomy of some important arboviruses

Family	Genus	Arboviruses
Togaviridae	Alphavirus	Chikungunya virus, O'nyong-nyong virus, Semliki Forest virus, Sindbis virus, Ross River virus, Eastern, Western and Venezuelan equine encephalitis viruses
Flaviviridae	Flavivirus	Japanese encephalitis virus, West Nile virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, Yellow fever virus, Dengue fever virus, tick-borne encephalitis virus, Kyasanur Forest Disease virus, Omsk hemorrhagic fever virus
Bunyaviridae	Bunyavirus	California encephalitis virus, Bunyamwera virus, La Crosse encephalitis virus
	Phlebovirus	Sandfly fever viruses (Toscana virus) Rift valley fever virus
	Nairovirus	Crimean-Congo hemorrhagic fever virus
	Hantavirus	Hantaan, Seoul, Puumala, Sin Nombre, New York and Black Creek Canal viruses
Reoviridae	Coltivirus	Colorado tick fever virus
	Seadornavirus	Banna virus
Arenaviridae	Arenavirus	Lassa virus, Junin, Machupo viruses, Lymphocytic choriomeningitis virus
Filoviridae	Filovirus	Ebola and Marburg viruses

Arboviruses are worldwide in distribution but are far numerous in the tropical countries. Most of them cause latent infections of wild animals but ~ 100 of them can infect humans. Arboviruses have a wide range of the natural hosts including many species of animals (monkeys, rodents, horses, small mammals, etc.) and birds. The ability to multiply in the arthropods is their special characteristic.

The most important arbovirus vectors are **mosquitoes** and **ticks**.

➢ The similar ecological group is **roboviruses** (**<u>ro</u>**dent-**<u>bo</u>**rne viruses). They are maintained in nature by direct transmission between rodents and sometimes infecting humans without participation of arthropod vectors. Roboviruses belong to different families (Arena-, Bunyaviridae, etc.); some of them are common with arboviruses.

➢ Most sensitive test-system for isolation of arboviruses/roboviruses is sucking white mice (intracerebral inoculation → fatal encephalitis). Also they can be cultivated in the yolk sac or CAM of chicken embryos and in cell cultures. Methods of virus indication are hemagglutination/hemadsorption (with goose erythrocytes), CPE (cell degeneration), plague formation, etc.

➢ Three antigens are important for arboviruses/roboviruses are hemagglutinins, complement fixing Ag and neutralizing Ag.

➢ Pathogenesis of arboviral infection: bite if insect vector → multiplication in monocytes/macrophages system → viremia. Then, in some cases, virus is transported to the target organs (CNS, liver, capillary endothelium).

➢ Arboviruses can cause following clinical forms: (1) **asymptomatic infection**; (2) **febrile illness** with or without rash and arthralgia/myalgia; (3) **hemorrhagic fever** (with hemorrhagic syndrome); (4) characteristic **systemic disease**; (4) **encephalitis**.

➢ Arboviral infections are zoonoses with a few possible exceptions (Dengue and yellow fever). Most arboviruses exist in nature in animal or avian species in which infection is asymptomatic. Human disease results only when the virus accidentally transferred to humans. The majority of arboviruses are transmitted by vector arthropod bites (transmissive mechanism); a few may also follow injection of infected cow's or goat's milk, inhalation of aerosols containing virus or contact with infected secretions or blood.

➢ Vector control measures, especially mosquito control, are essential to reducing the transmission of disease by arboviruses. Habitat control involves draining swamps and removal of other pools of stagnant water that often serve as breeding grounds for mosquitoes. *Insecticides* can be applied in rural and urban areas, inside houses and other buildings or in outdoor environments. People can also reduce the risk of getting bitten by arthropods by employing personal protective measures such as sleeping under mosquito nets, wearing protective clothing, applying insect *repellents* and (where possible) avoiding areas known to harbor high arthropod populations.

➢ Vaccines are available for the following arboviral diseases: Japanese encephalitis, tick-borne encephalitis, and yellow fever. Antibiotics are not an effective form of treatment and no effective antiviral drugs have yet been discovered. Treatment is supportive.

TOGAVIRUSES

CLASSIFICATION

Group: Group IV (+ssRNA)

Order: Unassigned

Family: Togaviridae

Genus: Rubivirus → **rubella virus**

Genus: Alphavirus → **Sindbis virus, Eastern equine encephalitis virus, Western equine encephalitis virus, Venezuelan equine encephalitis virus, Ross River virus, O'nyong'nyong virus, Chikungunya virus, Semliki Forest virus**.

MORPHOLOGY

Togaviruses are **enveloped**, have 70 nm in diameter, tend to be spherical (although slightly pleomorphic) viruses with **cubic capsid** and **positive sense, single-stranded RNA** genome.

ANTIGENIC STRUCTURE

- The alphaviruses show various degrees of antigenic *cross-reactivity* in these reactions (there are seven antigenic complexes, 30 species and many serotypes of alphaviruses).
- Envelope antigens are E1 and E2. These two glycoproteins are the targets of numerous serologic reactions including neutralization and hemagglutination/hemadsorption. **E2-Ag** is the main species-specific protective antigen and can be revealed in reaction neutralization (RN). Its function is adsorption of a virus to the target cells. **E1-Ag** is group-specific nonprotective hemagglutinin and can be revealed in RIHA. **C-Ag** (capsid antigen) is genus-specific antigen.

CULTIVATION

Alphaviruses can be cultivated in the sucking white mice (→ fatal encephalitis), chicken embryos (→ yolk sac inoculation and death within 2–3 days) and cell cultures (→ plague formation).

CAUSED DISEASES

There are many alphaviruses distributed around the world with the ability to cause human diseases. Once a human is bitten by the infected mosquito, the virus can gain entry into the bloodstream, causing *viremia*. The alphavirus can also get into CNS and this can lead to encephalitis, which can be fatal.

❖ The name "*Chikungunya*" is derived from the native word for the disease in which patient lies "doubled up" due to severe joints pain. Epidemics of **Chikungunya fever** have occurred in many African countries and Asia. Symptoms usually start with a sudden onset of malaise, fever and arthralgia. A maculopapular rash is common and some show hemorrhagic manifestations. Joint pain is typically lasting weeks or months but sometimes years. The fatality rate is low. During periods of epidemics humans are the reservoir of the virus. During other times, monkey, birds and other vertebrates have served as reservoirs.

Table 34 — Medically important arboviruses of genus Alphavirus

Virus	Human disease	Natural host (vector)	Distribution
Chikungunya virus	*Chikungunya fever*	Monkeys, birds, cattle, rodents; humans (mosquitoes)	Africa, Latin America, India, Asia
O'nyong'nyong virus	*O'nyong'nyong fever*	Primates, humans (mosquitoes)	Africa
Ross River virus	*Ross river fever*	Mammals, humans	Australia,

		(mosquitoes)	South Pacific
Semliki Forest virus	*Semliki Forest fever*	Birds (mosquitoes)	Africa
Eastern equine encephalitis virus	*Eastern equine encephalitis* (EEE)	Birds (mosquitoes)	Americas
Western equine encephalitis virus	*Western equine encephalitis* (WEE)	Birds, mammals (mosquitoes)	Americas
Venezuelan equine encephalitis virus	*Venezuelan equine encephalitis* (VEE)	Rodents, horses (mosquitoes)	Americas

❖ **Eastern equine encephalitis** is severe and sometimes lethal (fatality rate 30 %) infection of horses and humans. The most severe cases have dramatic onset of neurological symptoms leading to coma and death. **Western equine encephalitis** is less severe infection (fatality rate 4 %). Symptoms usually begin from headache, dizziness, fever, chills, and myalgia. **Venezuelan equine encephalitis** can either sustain an enzootic or epizootic life cycle. Enzootic subtypes have a smaller host range and are transmitted between reservoir rodent hosts by mosquitoes. The epizootic subtypes are responsible for most major human and equine (horse) epidemics. In horses, epizootic VEE virus has very high mortality (50–90 %). It causes fatal encephalitis. VEE in humans presents mostly as a flu-like illness. Disease mortality is less than 1 % and (encephalitic) only 14 % of cases involve CNS.

EPIDEMIOLOGY

- Source of alphaviral infections is zoonoses. Humans and horses are usually dead-end hosts or play a minor role in viral transmission (VEE virus is mainly amplified in horses). In most other cases the virus is maintained in nature in mosquitoes, rodents and birds.
- Mechanism of transmission is transmissive (vectors are mosquitoes).

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is long-lasting.
- There is no vaccines is available for common use.
- Vector control with repellents, protective clothing, breeding site destruction, and spraying are the preventive measures of choice.

Rubella virus

- **Rubella virus** is causative agent of **Rubella** ("*German measles*") which normally harmless childhood infection but can cause severe embryopathies in the first trimester of pregnancy (virus spreads via respiratory droplets and transplacental mechanism).
- Pathogenesis of rubella: infection is first initiated in the respiratory epithelium (→ catarrhal symptoms) and then spreads and replicates in the regional lymph nodes (especially posterior cervical lymph nodes) → viremia → dissemination of virus in the multiple sites. Maximal virus shedding from RT occurs 5–7 days before appearance of rash.
- Infection in childhood is likely to be asymptomatic. Symptoms of **postnatal rubella** are usually mild and consist of fever, catarrhal symptoms, lymphadenitis (cervical) and maculopapular rash.

• **Congenital rubella syndrome** (CRS) consists of the classical triad of bilateral cataract, microcephaly and sensorineural deafness (other features are thrombocytopenia, hepatosplenomegaly and purpuric rash). The risk of fetal malformation is highest (80 %) after maternal rubella in the first trimester of pregnancy.

• Congenital rubella confirmed by IgM detection in the fetal blood (this is an indication for medical abortion).

• Prevention of rubella is MMR. Booster doses of live attenuated monovaccine against rubella are recommended for boys and girls before puberty.

FLAVIVIRUSES

CLASSIFICATION

Group: Group IV (+ssRNA)
Order: Unassigned
Family: Flaviviridae
Genus: Hepacivirus → **hepatitis C virus** (HCV)
Genus Pegivirus → **hepatitis G virus** (HGV)
Genus: Flavivirus → **Yellow fever virus, Dengue fever virus, West Nile virus, Kyasanur Forest Disease virus, Omsk hemorrhagic fever virus, St. Louis encephalitis virus, Japanese encephalitis virus, Russian Spring Summer encephalitis virus**

MORPHOLOGY

Flaviviruses are **enveloped**, have 40-60 nm in diameter, spherical viruses with **cubic capsid** and **positive sense, single-stranded RNA** genome. The family gets its name from Yellow Fever virus, a type virus of Flaviviridae; *flavus* means yellow in Latin.

ANTIGENIC STRUCTURE

Antigenic structure is complex and resembles ones of Togaviridae (the most important antigens are E1-Ag, E2(H)-Ag and C-Ag).

CULTIVATION

Flaviviruses can be cultivated in the sucking white mice (→ fatal encephalitis), chicken embryos (→ yolk sac inoculation and death within 2–3 days) and cell cultures (→ plague formation).

CAUSED DISEASES

There are many flaviviruses distributed around the world with the ability to cause human diseases.

❖ **Japanese encephalitis** (JE) is severe illness with typically abrupt onset, fever, headache, and vomiting following with sigh of encephalitis (occipital rigidity, convulsions, mental retardation and coma). Fatality rate is 50 %. Domestic pigs and wild birds (herons) are reservoirs of the virus. Lifelong neurological defects such as deafness, emotional lability and hemiparesis may occur in those who have had CNS involvement. There are several JE vaccines are available.

❖ Approximately 80 % of **West Nile virus infections** in humans are subclinical. There are several clinical forms: (1) West Nile fever (WNF), in 20 % of cases, is a febrile syndrome that causes dengue-like illness with flu-like symptoms. (2) West Nile neuroinvasive disease (WNND), in < 1 % of cases, is when the virus infects CNS resulting in meningitis, encephalitis, meningoencephalitis or a poliomyelitis-like syndrome. West Nile encephalitis (WNE) is the most common neuroinvasive manifestation of WNND (prominent symptom is muscular weakness).

❖ **Kyasanur Forest Disease** (KFD, disease is also locally known as Monkey Disease/Monkey Fever) is a tick-borne viral hemorrhagic fever endemic to South Asia. The symptoms of the disease include a high fever with frontal headaches, myalgia, severe prostration followed by haemorrhagic symptoms (nasal bleeding, throat and gums bleeding, as well as gastrointestinal bleeding, hemorrhages into the skin). **Omsk HF** is clinically similar to KFD.

❖ **Tick-borne encephalitis (TBE)** often manifests as *meningitis, encephalitis,* or *meningoencephalitis*. TBE virus infects a range of hosts including ruminants, birds, rodents, carnivores, horses, dogs. Mechanisms of transmission are transmissive with ticks and (rare) through the non-pasteurized milk of infected cows/goats.

Three TBE virus epidemiological types are described:

(1) *European* TBE virus (Western or **S**pring **S**ummer **M**eningo**e**ncephalitis [**SSME**] virus); (2) *Siberian* TBE virus; (3) *Far-Eastern* TBE virus (formerly known as **R**ussian **s**pring **s**ummer **e**ncephalitis [**RSSE**] virus).

RSSE is the most serious form with high rate of fatality and permanent paralytic sequelae in some survivors. The disease is incurable once manifested, so there is no specific drug therapy for TBE. Prevention includes nonspecific (tick-bite prevention, tick checks) and specific prophylaxis in the form of inactivated RSSE vaccine for groups of risk.

❖ **Yellow fever** (historically known as *yellow jack* or *yellow plague*) is an acute viral disease which symptoms include fever, chills, nausea, muscle pain (particularly in the back), and headache. Symptoms typically improve within five days. In some people (15 % of cases) within a day of improving, the fever comes back, abdominal pain and jaundice occur with risk of hemorrhagic syndrome development and kidney failure (second "toxic" phase of a disease). The toxic phase is fatal in about 20 % of cases, making the overall fatality rate for the disease 3 %. In severe epidemics, the mortality may exceed 50 %.

Most cases are less severe, especially in the endemic areas and may present as undifferentiated fever without jaundice.

The epidemiology of yellow fever is complex. There are two epidemiological cycles. In the **urban cycle** humans are natural reservoir of infection and virus being transmitted by the domestic mosquitoes *Aedes aegypti*. In the **forest** (sylvatic, jungle) cycle wild monkeys act as reservoirs and forest mosquitoes (*A.africanus*) as a vectors. Human diseases occur only when humans come into the forest or when monkeys raid villages near the forest.

A safe and effective vaccine against yellow fever exists (*live attenuated vaccine 17D*) and some countries require vaccinations for travelers. Protection begins by the 10th day after vaccine use and lasts for at least 10 years (about 81 % of people are still immune after 30 years).

There is no specific therapy effective against the virus. It is common in tropical areas of South America and Africa (90 % of cases), but not in Asia. The yellow fever virus was the first human virus discovered.

▪ In 2009, the largest mass vaccination against yellow fever began in West Africa, specifically in Benin, Liberia, and Sierra Leone. According to the WHO "*mass vaccination cannot eliminate yellow fever because of the vast number of infected mosquitoes in urban areas of the target countries, but it will significantly reduce the number of people infected*". The WHO plans to continue the vaccination campaign in another five African countries — the Central African Republic, Ghana, Guinea, Côte d'Ivoire, and Nigeria. Also control of the yellow fever mosquito *A.aegypti* is of major importance, especially because the same mosquito can also transmit Dengue and Chikungunya fevers. *A.aegypti* breeds preferentially in water (in installations with drinking water supply, or in domestic waste; especially tires, cans, and plastic bottles). Two main strategies are employed to reduce mosquito populations. One approach is to kill the developing larvae (*larvicides*, larvae-eating fish and copepods). The second strategy is to reduce populations of the adult mosquito (insecticides, mosquito nets, etc.).

❖ **Dengue fever** (the name "*dengue*" is derived from the Swahili *Ki denga pepo*, meaning a sudden seizure by a demon; also known as *breakbone fever*) is a mosquito-borne tropical disease caused by the dengue virus. There are 5 serotypes of dengue fever virus: DENV-1, DENV-2, DENV-3 and DENV-4 (the fifth type was announced in 2013). Symptoms include fever, headache (typically located behind the eyes), conjunctival injections, lymphadenopathy; the alternative name for dengue, "breakbone fever", comes from the associated muscle and joint pains (especially in back and limbs) and a characteristic skin rash that is similar to measles.

In a small proportion of cases the disease develops into the life-threatening **dengue hemorrhagic fever (DHF)**, resulting in bleeding, low levels of blood platelets and blood plasma leakage, or into **dengue shock syndrome (DSS)**, where dangerously low blood pressure occurs. Infection with one serotype usually gives *lifelong immunity* to that type, but only *short-term immunity* to the others. Subsequent infection with a different serotype increases the risk of DHF/DSS. The most widely accepted hypothesis is that of **antibody-dependent enhancement** (ADE) of viral infection (antibodies after primary infection attach to the new serotype antigens but cannot neutralize them; this allows virus entrance to the endothelial cells via Fc-R resulting in DHF and/or DSS).

Children still carrying Ab (IgG) from their mother during the first year of life can also have these severe forms of infection due to the same mechanism.

Dengue is endemic in more than 110 tropical countries. This makes it one

of the most common vector-borne diseases worldwide. The WHO counts dengue as one of seventeen *neglected tropical diseases*.

There are two epidemiological cycles (like for yellow fever) — **urban** (predominant; human are reservoir of infection) and **sylvatic** (monkeys are reservoir of infection).

The main mechanism of transmission is transmissive by mosquitoes (*Aedes aegypti*). Also dengue can be transmitted via infected blood products and through organ donation; vertical transmission has been reported. There are no approved vaccines for the dengue fever. Prevention thus depends on control of and protection from the bites of the mosquito. Treatment of dengue is supportive (oral or intravenous rehydration).

Table 35 — Medically important arboviruses of genus Flavivirus

Virus	Human disease	Natural host (vector)	Distribution
Yellow fever virus	*Yellow fever* (hepatitis, kidney failure)	Monkeys, humans (mosquitoes)	Africa, South America
Dengue fever virus	*Dengue fever* (rash, arthralgia, myalgia)	Monkeys, humans (mosquitoes)	Tropics
West Nile virus (WNV)	*West Nile virus infections*	Birds, mammals (mosquitoes)	Africa, Asia
Kyasanur Forest Disease virus	*Kyasanur Forest Disease*	Rodents, mammals, birds (ticks)	India (Karnataka)
Omsk hemorrhagic fever virus	*Omsk hemorrhagic fever*	Small mammals (ticks)	Russia
St. Louis encephalitis virus	*St. Louis encephalitis*	Birds (mosquitoes)	Americas
Japanese encephalitis virus	*Japanese encephalitis*	Birds, pigs (mosquitoes)	East Asia (Korea, Japan, India)
Tick-borne encephalitis virus	*RSSE SSME*	Rodents, cows/goats (ticks)	Russia, East Europe

EPIDEMIOLOGY

- Source of flaviviral infections is zoonosis. Humans are usually dead-end hosts or play a minor role in viral transmission (exceptions are urban cycles of yellow and dengue fevers).
- Mechanism of transmission is transmissive (vectors are mosquitoes, ticks).

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is long-lasting.
- Vaccines are available for yellow fever, JE and TBE.
- Vector control with repellents, protective clothing, breeding site destruction, and spraying are the preventive measures of choice.

ARENAVIRUSES

CLASSIFICATION

Group: Group V (-ssRNA)

Order: Unassigned
Family: Arenaviridae
Genus: Arenavirus

Arenaviruses are divided into two groups: **Old World** (found in the Eastern Hemisphere in places such as Europe, Asia, Africa) and **New World** viruses (found in the Western Hemisphere, in places such as Argentina, Bolivia, Venezuela, Brazil, and USA). The differences between these groups are distinguished geographically and genetically. Because of the epidemiological association with rodents arenaviruses are designated as **roboviruses**.

- **LCMV-Lassa virus (Old World) complex** → ***L****ymphocytic* ***c****horio****m****eningitis* ***v****irus* (LCMV) and Lassa virus. LCMV is the only arenavirus to exist in both areas but is classified as an Old World virus.
- **Tacaribe virus (New World) complex** → *Guanarito virus, Junin virus, Machupo virus, Sabiá virus* and others.

MORPHOLOGY

- Arenaviruses show *grainy particles* in their envelope under the electron microscopy that are ribosomes acquired from their host cells (name *Arenosus* came from the Latin meaning *sandy*).
- Arenaviruses are spherical, **enveloped** viruses with a diameter of 120 nm. The virus contains a "beaded", **helical** capsid with two **single-stranded RNA** segments → small (S) and large (L). Although they are often miscategorized as negative sense viruses, they are in fact **ambisense** (meaning they possess genomic elements with minus/antisense as well as plus/sense polarity).

ANTIGENIC STRUCTURE

The S-segment RNA encodes the viral nucleocapsid protein (NP) and glycoprotein (GP). The L-segment RNA encodes the viral RNA-dependent RNA-polymerase (L) and a small RING-domain containing protein (Z).

- Z-Ag is essential for assembly and budding of arenaviruses.
- GP-Ag is essential for fusion, assembly and budding of arenaviruses.

CULTIVATION

Arenaviruses can be cultivated in the rodents and cell cultures.

CAUSED DISEASES

❖ **LCM** viruses cause influenza-like febrile illness, but occasionally they may cause *meningitis*, characteristically accompanied by large numbers of lymphocytes in the CSF. LCM manifests itself in a wide range of clinical symptoms, and may even be asymptomatic for immunocompetent individuals. For immunocompetent mothers, there is no significant threat, but the virus has damaging effects upon the fetus. If infection occurs during the first trimester, LCMV results in an increased risk of spontaneous abortion. Later congenital infection may lead to malformations such as intracranial calcifications, microcephaly or macrocephaly, mental retardation and seizures. Other findings include chorioretinitis, which is followed by chori-

oretinal scarring, is the most common ocular lesion. Mortality among infants is approximately 30 %. Among the survivors, two thirds have neurologic abnormalities.

❖ **Lassa fever** is endemic in West Africa. The virus was first isolated from Americans stationed in the village of *Lassa,* Nigeria. The virus can be transmitted person-to-person (airborne route or with direct contact with infected human blood, urine, or semen). Transmission through breast milk has also been observed. Healthcare stuff must wear special clothing, gloves and facemasks. In 80 % of cases, the disease is asymptomatic, but in the remaining 20 %, it takes a complicated course. Lassa fever is characterised by high fever, severe myalgia, haemorrhagic skin rash, and occasional visceral haemorrhage as well as necrosis of liver and spleen. The other symptoms include nausea, bloody vomiting and diarrhea, stomach ache, cardiovascular problems, cough, chest pain, seizures, etc. Clinically, Lassa fever infections are difficult to distinguish from other viral hemorrhagic fevers such as Ebola and Marburg, and also malaria. About 15–20 % of hospitalized Lassa fever patients will die from the illness. However during epidemics mortality can reach 50 %. The mortality rate is greater than 80 % when it occurs in pregnant women during third trimester.

Table 36 — Medically important arenaviruses

Virus	Human disease	Natural host (rodent)	Distribution
Lymphocytic choriomeningitis virus	*Lymphocytic choriomeningitis*	House mouse	Worldwide
Lassa virus	*Lassa fever*	Natal multimammate mouse	West Africa
Junin virus	*Argentine hemorrhagic fever*	Kind of mouse	Argentina
Machupo virus	*Bolivian hemorrhagic fever*	Kind of mouse	Bolivia
Guanarito virus	*Venezuelan hemorrhagic fever*	Kind of mouse	Venezuela
Sabiá virus	*Brazilian hemorrhagic fever*	Kind of mouse	Brazil

EPIDEMIOLOGY

- Source of arenaviral infections is zoonosis (rodents). Each virus usually is associated with a particular rodent host species in which it is maintained. Humans are usually dead-end hosts or play a minor role in viral transmission.
- Mechanism of transmission is contact (with rodents). Humans can be infected through mucosal exposure to aerosols (sprays of rodent dried excreta, especially urine that is dropped in the environment), or by direct contact of abraded skin with the infectious material, derived from infected rodents; also fecalo-oral.

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is long-lasting.

- There is no vaccines is available for common use.
- Rodents control is the preventive measure of choice.

BUNYAVIRUSES

CLASSIFICATION

Group: Group V (-ssRNA)
Order: Unassigned
Family: Bunyaviridae

(1) Genus: Orthobunyavirus → type species is **Bunyamwera Virus, California encephalitis virus, La Crosse virus**

(2) Genus: Phlebovirus → **Phlebotomus fever** or **sandfly fever viruses** (*Naples* virus, *Rift Valley fever* virus, *Sicilian* virus, *Toscana* virus, etc.). Recently identified is another human pathogenic serotype — **SFTS virus** (**s**evere **f**ever with **t**hrombocytopenia **s**yndrome described in China)

(3) Nairovirus → **Crimean-Congo hemorrhagic fever virus**

(4) Hantavirus → HFRSVs (**h**emorrhagic **f**ever with **r**enal **s**yndrome **v**iruses) are included **Hantaan, Dobrava-Belgrade, Seoul** and **Puumala**, viruses. **Sin Nombre** virus → Hantavirus pulmonary syndrome.

MORPHOLOGY

Bunyaviruses are spherical, **enveloped** viruses with a diameter of 100 nm with **helical** capsid and triple segmented **negative sense single-stranded RNA** → small (S), medium (M) and large (L) segments.

Bunyavirus is so named from the type species *Bunyamwera* virus isolated in Uganda in 1946.

ANTIGENIC STRUCTURE

The L-segment encodes RNA-polymerase, necessary for viral RNA replication and mRNA synthesis. The M-segment encodes the viral type-specific glycoproteins (**Gn** and **Gc**), which aid the virus in attaching to and entering the host cell (they are also hemagglutinins). The S-segment encodes the nucleocapsid group-specific protein (**N-Ag**).

CULTIVATION

Bunyaviruses can be cultivated in the chicken embryos, rodents and cell cultures.

CAUSED DISEASES

❖ **Crimean–Congo hemorrhagic fever** (CCHF) is a widespread tick-borne viral disease, a zoonosis of domestic and wild animals that may affect humans. CCHFV is first isolated in Crimean in 1945 and then found to be identical with Congo fever virus isolated in Congo (Zaire). Typically, after tick bite (also blood of patients is highly contagious and direct transmission may occur through contact) flu-like symptoms appear, which may resolve after one week. In up to 75 % of cases signs of hemorrhage appear within 3–5 days of the onset

of illness (nasal, intestinal bleedings, DIC-syndrome, etc.). Fatality rate is 30 %.

❖ **Hemorrhagic fever with renal syndrome** (HFRS, also known as *Korean hemorrhagic fever*, *rodent-borne nephropathy*). The species that cause HFRS include Hantaan, Dobrava-Belgrade, Seoul and Puumala. It is found in Europe, Asia and Africa. Initial symptoms include intense headaches, back and abdominal pain, fever, chills, nausea and blurred vision. Later symptoms can include low blood pressure, acute shock, vascular leakage and acute kidney failure, which can cause severe fluid overload.

Table 37 — Medically important bunyaviruses

Virus	Human disease	Natural host (vector)	Distribution
Arboviruses			
Genus **Orthobunyavirus** (California encephalitis virus)	*California encephalitis*	Rodents (mosquitoes)	North America
Genus **Phlebovirus** (Naples virus, Sicilian virus and Toscana virus)	*Pappataci fever* (also known as Phlebotomus fever)	Unknown, small mammals? (phlebotomine sandflies)	Southern Europe, North Africa, the Balkans, India, etc.
Genus **Phlebovirus** (Rift Valley fever virus)	*Rift Valley fever*	Cattle, sheep (mosquitoes)	Africa (Kenya)
Genus **Nairovirus** (Crimean-Congo hemorrhagic fever virus)	*Crimean-Congo hemorrhagic fever (CCHF)*	Small mammals (ticks)	Russia, Central Asia, Africa
Roboviruses			
Genus **Hantavirus** (Hantaan virus)	*Hemorrhagic fever with renal syndrome (HFRS)*	Rodents	Europe, Korea, Africa
Sin Nombre virus	*Epidemic neuropathy* *Hantavirus pulmonary syndrome (HPS)*	Deer mice	North, Central and South America

The course of the illness can be split into five phases: febrile phase (redness of cheeks and nose, fever, chills, diarrhea, headache, nausea, abdominal and back pain, catarrhal symptoms); hypotensive phase (blood platelet levels drop and symptoms can lead to tachycardia and hypoxemia); oliguric phase (onset of renal failure and proteinuria); diuretic phase (diuresis); convalescent phase. HFRS can also be fatal. In some cases, it has been known to cause permanent renal failure.

Transmission by aerosolized rodent excreta still remains the only known way the virus is transmitted to humans. Rodent control in and around the home remains the primary prevention strategy, also wear a mask to avoid inhalation of aerosolized rodent secretions.

❖ **Epidemic neuropathy** is a type of HFRS caused by the Puumala virus → sudden onset with fever, abdominal pain, headache, back pain and gastrointestinal

symptoms; more severe symptoms include internal hemorrhaging. It is generally milder than the HFRS and distributed in Scandinavia.

❖ **Hantavirus pulmonary syndrome** (HPS) is an often fatal pulmonary disease. In USA the causative agent is the *Sin Nombre virus* carried by deer mice. Prodromal symptoms include flu-like symptoms such as fever, cough, myalgia, headache and lethargy. It is characterized by a sudden onset of shortness of breath with rapidly evolving pulmonary edema that is often fatal (fatality rate of 36 %).

EPIDEMIOLOGY

• Source of infections is zoonosis (mammals, rodents, etc.). Domestic rats appear to be the source of infection in urban cases of HFRS. Humans are usually dead-end hosts or play a minor role in viral transmission.

• Mechanisms of transmission are transmissive (for arboviruses) and contact (for roboviruses). Most bunyaviruses are mosquito-borne but some transmitted by sandflies (Pappataci fever) or ticks (Crimean-Congo hemorrhagic fever). Hantaviruses normally infect rodents and do not cause disease in these hosts (**roboviruses**). Humans may become infected with hantaviruses through contact with rodent urine, saliva, or feces (for example, inhalation of the virus aerosols in dried rodent feces or urine). Hantavirus is named for the *Hantan River* area in South Korea where an early outbreak was observed.

IMMUNITY, PREVENTION AND TREATMENT

• Immunity is long-lasting.
• There is no vaccines is available for common use.
• Rodents and arthropod control is the preventive measure of choice.

FILOVIRUSES

CLASSIFICATION

Group: Group V (-ssRNA)
Order: Mononegavirales
Family: Filoviridae
Genus: Ebolavirus

The members of this genus are called **ebolaviruses**. The five known virus species are named for the region where each was originally identified: **Bundibugyo** ebolavirus, **Reston** ebolavirus, **Sudan** ebolavirus, **Tai Forest** ebolavirus (originally Côte d'Ivoire ebolavirus) and **Zaire ebolavirus** (type species).

Genus: Marburgvirus → species: **Marburg virus** (MARV) and **Ravn virus** (RAVV). Both viruses cause *Marburg virus disease* in humans and nonhuman primates, a form of viral hemorrhagic fever.

MORPHOLOGY

Filoviruses are pleomorphic particles, appearing as long **filamentous threads** or **U-like** shaped virions with length from 700 nm (Marburg virus) to

800 nm (Ebola virus). It is **complex** viruses with **helical** capsid and **single-stranded, nonsegmented, negative-sense RNA**.

ANTIGENIC STRUCTURE

- Structural proteins of nucleocapsid are **NP** (nucleoprotein), **VP30** (co-factor of viral polymerase), **VP35** (phosphoprotein) and **L**-protein (RNA-dependent RNA-polymerase).
- Structural proteins of envelope are **VP24** and **VP40** (matrix proteins); also surface **GP**-complex (GP1 and GP2) is forming the viral spikes.

CULTIVATION

Filoviruses can be cultivated in the cell cultures.

CAUSED DISEASES

❖ **Ebola virus disease** (EVD) also known as **E**bola **h**emorrhagic **f**ever (EHF), or simply Ebola, is a disease of humans and other primates caused by ebolaviruses. It is a type of hemorrhagic fever having a very high case fatality rate 25–90 % in humans of all the viral hemorrhagic fevers. Causative agents are four species of ebolaviruses — Bundibugyo, Sudan, Tai Forest and Zaire; the fifth, Reston virus, has caused EVD only in primates.

Symptoms of EHF typically start after 21 days of incubatory period as a fever, sore throat, muscle pain, and headaches (sudden influenza-like stage). Then, vomiting, diarrhea and rash usually follow, along with decreased function of the liver and kidneys. In about half of the cases, the skin may develop a maculopapular rash. In some cases hemorrhagic syndrome occurs (vomiting of blood, coughing up of blood, or the presence of blood in stool, etc.). In general, bleeding often indicates a worse outcome, and this blood loss may result in death. The mean time to death from the onset of symptoms is 7–8 days. Those who survive often have ongoing muscle and joint pain, liver inflammation, decreased hearing, and may have constitutional symptoms such as continued weakness, decreased appetite, etc.

Filovirus infections appear to be immunosuppressive. Fatal cases often show impaired humoral immune responses.

Ebolaviruses were first described after outbreaks of EVD in southern Sudan in June 1976 and in Zaire in August 1976. The name Ebolavirus is derived from the *Ebola River* in Zaire (now the Democratic Republic of the Congo). Ebolaviruses are closely related to marburgviruses.

➢ **Zaire ebolavirus** (ZEBOV) has the highest mortality rate among all ebolaviruses (up to 90 % in epidemics). There have been more outbreaks of Zaire ebolavirus than of any other species. The virus is responsible for the 2014 West Africa outbreak, with the largest number of deaths to date.

➢ **Sudan ebolavirus** (SUDV): it was at first assumed to be identical with ZEBOV. SUDV is believed to have broken out first amongst cotton factory workers in Nzara, Sudan (now in South Sudan), in June 1976, with the first case

reported as a worker exposed to a potential natural reservoir which is still unknown. The fatality rate is for SUDV 50–70 %.

➢ **Tai Forest ebolavirus** (TAFV) was first discovered among chimpanzees from the Tai Forest in Côte d'Ivoire, Africa (1994). Studies of tissues taken from the monkey showed results similar to human cases during the 1976 Ebola outbreaks in Zaire and Sudan. The source of the virus was believed to be the meat of infected western red colobus monkeys. TAFV is also virulent for humans.

➢ **Bundibugyo ebolavirus** (BDBV) caused outbreak of Ebola in the Bundibugyo District (Uganda, 2007) with mortality rate of 34 %.

➢ **Reston ebolavirus** (RESTV) was discovered during an outbreak of simian hemorrhagic fever virus in 1989. *This virus cannot cause EVD in exposed humans.*

❖ **Marburg virus disease** (MVD) was recognized in 1967 among laboratory workers exposed to tissues of African green monkeys imported into Germany (*Marburg*). Transmission from patients to medical personnel occurred, with high mortality rates. Outbreaks have been documented in Kenya, South Africa, Democratic Republic of the Congo, and most recently, in 2005, in Angola. Marburg virus can infect guinea pigs, mice, hamsters, monkeys. Clinical symptoms of MVD are indistinguishable from EVD.

EPIDEMIOLOGY

- Source of infection is zooanthroponosis (fruit bats are believed to be the natural reservoir of Ebola, also monkeys, domestic dogs and pigs). Although monkeys are not considered to be reservoir hosts as most infected animals die too rapidly to sustain virus survival.
- Mechanisms of transmission direct contact with body fluids, such as blood of an infected human or animals and upon contact with a recently contaminated item or surface, like needles or syringes (most people spread the virus through blood, feces and vomit). Entry points for the virus include the nose, mouth, eyes, open wounds, cuts and abrasions. Spread of the disease through the air between primates, including humans, has not been documented in either laboratory or natural conditions. Semen or breast milk of a person with EVD after recovery may still carry the virus for several weeks to months.
- EVD outbreaks typically occur intermittently in tropical regions of sub-Saharan Africa. Through 2013, WHO reported a total of 1,716 cases in 24 outbreaks. The largest outbreak to date is the ongoing epidemic in West Africa, which is centered in Guinea, Sierra Leone and Liberia. This outbreak has 19,028 reported cases resulting in 7,363 deaths (on December, 2014).

IMMUNITY, PREVENTION AND TREATMENT

- Immunity is believed to be long-lasting (~10 years), but resistance to reinfections is unclear.
- No specific treatment or vaccine for the virus is commercially available, although a number of potential treatments are being studied. Treatment is supportive (oral or intravenous rehydration therapy).

• Health-care workers treating those who are infected are at greatest risk of getting infected themselves. The use of isolation facilities in hospital settings remains the most effective means of controlling EVD. Strict barrier nursing techniques should be implemented. Extreme care must be taken with infected blood, secretions, tissues and wastes.

• People who have recovered are not infectious, but dead bodies remain infectious; thus, people handling human remains in practices such as traditional burial rituals or more modern processes such as embalming are at risk.

• Personnel involved in the transportation and care of nonhuman primates should be instructed about the potential hazards of handling such animals.

RHABDOVIRUSES

CLASSIFICATION

Group: Group V (-ssRNA)

Order: Mononegavirales

Family: Rhabdoviridae

Genus: Lyssavirus → species: **Rabies virus,** also antigenically- and genetically-related rabies-like viruses: *Lagos virus, Mokola virus, Duvenhage virus, Kotonkan virus, European bat Lyssavirus* and *Australian bat Lyssavirus*.

Genus: Vesiculovirus → species: **Vesicular stomatitis viru**s (naturally it is arbovirus of animals; in laboratory — *indicator virus* used in phenomenon of interference in cell cultures)

MORPHOLOGY

• Rhabdoviruses are **enveloped** and have a **single stranded RNA** genome with **negative-sense** and **helical capsid**.

• The rabies virus has a bullet like shape (180 nm), one end is rounded (conical) and the other end is planar (concave). The envelope carries knob-like spikes composed of gpG. Spikes do not cover the planar end of the virion. Beneath the envelope is the membrane or matrix (M) protein layer which may be invaginated at the planar end. The membrane may project outwards from the planar end of some virions forming a bleb.

ANTIGENIC STRUCTURE

There are five proteins: large protein (L), glycoprotein (G), nucleoprotein (N), phosphoprotein (P) and matrix protein (M). Specific regions of the G protein have been shown to be most antigenic in leading to the production of virus neutralizing antibodies.

CULTIVATION

Rhabdoviruses can be cultivated in the cell cultures and laboratory animals.

• Experimental infection can be produced in any laboratory animal but **mice** are the animals of choice. They can be infected by any route. After intracerebral inoculation they develop encephalitis and die.

• Rabies viruses may be categorized as either *fixed* (adapted by multiple

passages in animals or cell cultures) or *street* (wild type). Intracytoplasmic inclusion bodies (**Negri bodies**) can be demonstrated in the brain of animals dying from street virus infection. The fixed virus is more neurotropic and Negri bodies are usually not demonstrable in the brain of animals dying from fixed virus infection. The fixed virus is used for vaccine production.

CAUSED DISEASES

Rabies is a viral disease that causes acute inflammation of the brain in humans and other warm-blooded animals.

• *Pathogenesis of rabies*: from the wound of entry rabies virus appears to multiply in the muscles and connective tissue for 48–72 hours → virus travels quickly along the neural pathways of PNS (gpG is binding with *acetylcholine receptors* and virus is endocytosed by neurons) → *retrograde axonal centripetal transport* of the virus to CNS (brain → encephalitis and beginning of symptoms, also may be affected spinal cord → myelitis) through the synapses of the neurons → virus further centrifugally along the nerve trunks spreads to other organs (*salivary glands*, *cornea*, *facial skin*) → it multiplies in salivary glands and is shed by saliva. Viremia is not clinically significant. In nerve system virus produces degeneration of motor neurons.

• Five general stages of rabies are recognized in humans: incubation, prodrome, acute neurologic period, coma, and death. Once the patient becomes symptomatic, treatment is almost never effective and mortality is over 99 %.

The incubation period in rabies, is usually lasting 30 to 90 days but ranging from as few as 5 days to longer as 2 years after initial exposure. Incubation periods depending on the location (proximity to the brain) and severity of the contaminated wound and the amount of virus introduced (may be shorter in individuals bitten close to CNS; ex.: head, neck). Clinical symptoms are first noted during the prodromal period, which usually lasts from 2 to 10 days. These symptoms are often nonspecific (general malaise, fever, and fatigue) or suggest involvement of RT (sore throat, cough, and dyspnea), GIT (anorexia, dysphagia, nausea, vomiting, abdominal pain, and diarrhea), or CNS (headache, anxiety, irritability, insomnia, depression, excessive libido and nervousness). An early symptom is often pain or paresthesia at the site of virus entry (ex.: place of healed wound).

The acute neurologic period begins with objective signs of CNS dysfunction. The disease may be classified as *furious rabies* if hyperactivity predominates (80 % of cases) and as *dumb rabies* (20 %) if paralysis dominates the clinical picture (fever, nuchal rigidity, muscle spasms, convulsions, hyperventilation, and hypersalivation may occur in both forms of the disease). Hyperactivity is characterized by bouts of bizarre behavior, agitation or seizures appearing between apparently normal periods. The pathognomonic feature of furious rabies is difficulty in drinking ("**hydrophobia**" is ancient name of human rabies) together with intense thirst. Attempts to drink bring painful spasms of the pharynx and larynx producing chocking or gagging that patient develops even during the sight or sound of water. Generalized convulsions and coma follow. Death usually occurs within 1–6 days due to respiratory arrest during convulsions.

Although life support measures can prolong the clinical course of rabies, rarely will they affect the outcome of disease. The possibility of recovery, however, must be recognized, and when resources permit, every effort should be made to support the patient. Only few cases of human "recovery" have been documented after showing symptoms. These were with extensive treatment known as the *Milwaukee protocol* (chemically induced coma and administering antiviral drugs, 2004).

The disease can only be diagnosed after the start of symptoms.

Some persons exposed to real or imaginary risk of rabies develop a psychological disorder which has been called *lyssaphobia* (hydrophobiophobia). Patients present with anxiety, irritability and exaggerated hydrophobia (they are afebrile). Sedation helps them.

EPIDEMIOLOGY

- Source of is global zoonosis. Cattle, cats and foxes are highly sensitive to rabies, whereas skunks, opossums and fowl are relatively resistant. Humans and dogs (also wolves) occupy an intermediate position. In the Americas, bat bites are the most common source of rabies infections in humans. Rodents are very rarely infected with rabies.

Natural reservoirs of rabies are mustelids, viverrids, ermine, skunk, mink, pole cats in Russia and Africa, mongoose in Asia, etc. (they need for survival of virus in latent state) → wild animals (diseased "vectors" like foxes, wolves, jackals) → domestic animals and/or humans.

- Rabies can be transmitted when an infected animal scratches or bites another animal or human. Saliva from an infected animal can also transmit rabies if the saliva comes into contact with a mucous membrane of another animal or human (licked by an animal). Most rabies cases in humans are the result of dog bites. In furious rabies of dogs (much more common than dumb rabies) they run amok, biting without provocation and indiscriminately (also dogs can exhibits *uncharacteristic behavior*). The lower jaw droops and saliva drools from the mouth. In dumb rabies animal lies huddled, unable to feed (animal may not bite but attempts to feed it are dangerous). *This is an example of a viral pathogen modifying the behavior of its host to facilitate its transmission to other hosts.* Rabies dogs usually die in 3–5 days (incubation period is usually 3–6 weeks but it may range from 10 day to a year). The observation period of 10 days is recommended for apparently healthy animals because the virus may be present in the saliva 3–4 days before onset of rabies. Rabies in cats is similar to canine rabies. In wild animals the chief characteristic of rabies is loss of fear of man and other animals.

- Human rabies is a dead end. Direct person-to-person transmission of rabies has not been recorded though the virus present in saliva. An unusual mode of transmission of rabies has occurred on some recipients of corneal grafts (from donor with unsuspected rabies).

- Pneumotropic rabies virus strains have been obtained from bats. Humans may be infected by aerosols if they enter caves colonized by infected bats.

- The risk of rabies is highest in countries with hyperendemic canine ra-

bies, including most of Asia (India is endemic for rabies), Africa, and Latin America (after 1996 only Antarctica is free from rabies viruses). Two epidemiological types of rabies exist – **urban** (transmitted by domestic animals) and **sylvatic** (involved wild animals).

• Rabies-like viruses: *Lagos bat virus* is isolate from Lagos Island, Nigeria; *Mokola virus* is isolated from many domestic and wild animals in Africa; Duvenhage virus is isolated from brain of man who died from clinical rabies in South Africa after being bitten by a bat. Australia was considered free of rabies and related viruses till 1996, when a Lyssavirus was isolated from frugivorous bats. The relevance of rabies related viruses with human diseases is unclear though some of them have caused illness and death in humans.

IMMUNITY, PREVENTION AND TREATMENT

• No specific antirabies agents are useful once clinical signs or symptoms develop. Specific prophylaxis is generally employed after exposure to infection and is therefore called **antirabic treatment**.

• Animal rabies is prevented by vaccinating of susceptible species, particularly dogs and cats.

• Human rabies is best prevented by avoiding exposures to the disease. If treatment (postexposure prophylaxis) is necessary, it should be initiated promptly (if untreated about half of animal bites may develop rabies). **Postexposure prophylaxis** consists of the combination of *local wound cleansing*, *rabies vaccine* and *passive immunization*. Postexposure treatment will abort the infection, but there is no cure for clinical disease.

• The wound should be immediately scrubbed well with soap and water, after that treated with **antiseptics** (quaternary ammonium compounds, tincture or aqueous solution of iodine, or alcohol 40–70 %).

• There are two currently commonly licensed in many countries vaccines are the ***h****uman* ***d****iploid* ***c****ell* ***v****accine* (HDCV) and ***r****abies* ***v****accine* ***a****dsorbed* (RVA). Vaccination (ex.: HDCV) requires 5 or 6 doses (on 0, 3, 7, 14, 30 and/or 90 day). This course is expected to give protection for at least five years.

• Passive immunization can be done by several preparations: *antirabic horse serum purified equine rabies immune globulin* (ERIG) and ***h****uman* ***r****abies* ***i****mmune* ***g****lobulin* (HRIG). HRIG is free from risk of anaphylactic shock, but has risk of HIV/HBV/HCV-infections and much costlier than ERIG. Passive immunization should be employed whenever the exposure is considered of high risk. It must be given before or simultaneously with the first dose of vaccine, but not after it.

• **Preexposure immunization** may be offered to persons at high risk, such as veterinarians, animal handlers, certain laboratory workers, and persons spending time (e.g., 1 month or more) in foreign countries where rabies is a constant threat.

Lecture 20. RETROVIRUSES. DNA-VIRUSES

RETROVIRUSES

CLASSIFICATION

Group: Group VI (ssRNA-RT)
Order: Unassigned
Family: Retroviridae

Subfamily: Orthoretrovirinae	Subfamily: Spumaretrovirinae
Genera: Alpha-, Beta-, Delta-, Epsilon- and Gammaretrovirus	Genus: Spumavirus
Genus: **Lentivirus** → HIV	Species: simian foamy virus and **human foamy virus** (HFV)

Species "foamy viruses" derive their name from the characteristic "*foamy*" appearance of CPE induced in the cells. HFV has been isolated from patients with various neoplastic and degenerative diseases (myasthenia gravis, multiple sclerosis, etc.) but the virus' etiological role is still unclear. Orthoretroviruses genera include many oncogenic animal and avian viruses (avian leukosis virus, feline leukemia virus, etc.). Genus Deltaretrovirus includes the cancer-causing **human T-lymphotropic virus** (HTLV). There are four types of HTLVs have been identified. HTLV-I and HTLV-II are associated with adult *T-cell leukemia/lymphoma*.

Genus *Lentivirus* includes **human immunodeficiency virus** (HIV); others include simian, feline immunodeficiency viruses, equine infectious anemia virus, visna/maedi viruses (slow animal infections viruses).

First indication began in 1981 when reports from New York and California, USA of a sudden increase in incidence of two very rare diseases — Kaposi sarcoma and *Pneumocystis carinii* pneumonia (mycosis). These diseases were detected in young adults who are homosexual and drug addicts. They appeared to have lost their immune competence rendering them to overwhelming and fatal infections causing by relatively avirulent microorganisms. This condition was named **AIDS** (**a**cquired **i**mmune **d**eficiency **s**yndrome). Isolation of etiological agent was first reported in 1983 by French physician, *Luc Montagnier* and his colleagues from the Pasteur institute in Paris. This group isolated a retrovirus from the patients who came back from West Africa. These patients had persistent generalized lymphadenopathy, which is

the main manifestation of AIDS. In 1984, American physician of National Institute of Health under leadership of Robert Gallo reported isolation of a retrovirus from AIDS patients. Robert Gallo called this virus human T-cell lymphotropic virus type III (HTLV-III). In 1986, International Committee on Virus Nomenclature decided to name this virus as Human Immunodeficiency Virus (HIV).

MORPHOLOGY

• Retroviridae is a family of enveloped viruses that replicate in a host cell through the process of **reverse transcription**. In most viruses, DNA is transcribed into RNA, and then RNA is translated into protein. However, retroviruses function differently — their RNA is reverse-transcribed into DNA, which is integrated into the host cell's genome (when it becomes a **provirus**) and then undergoes the usual transcription and translational processes to express the genes carried by the virus. So, the information contained in a retroviral gene is used to generate the corresponding protein via the sequence: RNA → DNA → RNA → polypeptide. Existence of HIV in a form of provirus underlies of long *latent HIV-infection* (time to time *lytic infection* is initiated releasing progeny virions which infect other cells).

• HIV is spherical **enveloped** virus (90–120 nm). Capsid has an *outer cubic shape shell* and an *inner cones-shaped core ("conical capsid") e*nclosing the genome. Genome of HIV is **diploid**. It composed of 2 identical **single stranded positive RNA**. The envelope has projecting **knob-like spikes** (gp120) on the surface and the **anchoring transmembrane pedicles** (gp41). The spikes are major surface components of the virus which binds to CD4 receptors of the susceptible host cells (transmembrane pedicles cause fusion). This is primarily ***CD4+ T-lymphocytes*** (T-helpers). Some other immune cells also possess CD4 and so are sensitive to HIV (5–10 % of *B-lymphocytes*, 10–20 % of *monocytes* and *macrophages*, including Langerhans cells in dermis and alveolar macrophages). *Glial cells* and *microglia* in CNS are also susceptible. *Follicular dendritic cells* from tonsils can be infected by HIV without involvement of CD4.

• Binding with CD4 is not by itself enough for entry of virus. This requires participation of *coreceptor molecules* (chemokine coreceptors) such as CXCR4 for T-cell tropic HIV strains (**T-tropic** or **X4 strains**) and CCR5 for macrophage tropic strains (**M-tropic** or **R5 strains**). Person with mutation of CCR5 are highly resistant to HIV. Cell fusion is induced by X4 strains (syncytial formation occurs late in HIV-infection and associated with progression to AIDS). R5 do not induce syncytia and present early in the course of infection.

• The genome of HIV contains **structural genes** (gag, pol, env) and **regulatory genes**. The product of these genes act as antigens.

Table 38 — Important HIV genes and antigens

Gene	Product	Function
Structural genes (→ structural proteins)		
gag	p24	Capsid protein

Gene	Product	Function
	p17	Matrix protein
	p7, p9	Core nucleocapsid proteins
env	gp120	Surface protein that binds to CD4 of host cells
	gp41	Transmembrane protein for fusion with host cells
pol	RT	Production of virus dsDNA
	Integrase	Integration of virus dsDNA into host DNA (formation of *provirus*)
	Protease	Cleaving of polyproteins (ex.: gp160 → gp120 and gp41)
Regulatory genes		
LTR	DNA **l**ong **t**erminal **r**epeats	Integration of virus into a host genome
tat	Transactivator of virus genes	Transactivator of transcription ("upregulation" or enhancing the expression of all viral genes)
rev	Regulator of virus genes	Enhancing the expression of structural proteins
vif	Viral infectivity gene	Influencing on infectivity of virions
nef	Negative factor gene	Down regulating viral replication
vpu	Presents in HIV-1	Enhancing release of viral progeny
vpx	Presents in HIV-2	
vpr	-	It is required for virus replication in nondividing cells (macrophages) Induces apoptosis in proliferating cells

ANTIGENIC STRUCTURE

• **Major antigens of HIV**:

(1) Envelope antigens (spike A): **gp120** (principal envelop Ag for binding with CD4 receptors of host cell); transmembrane **gp41** (fusion with host cell).

(2) Matrix protein – **p17 (p18)**.

(3) Principal core Ag — **p24** (can be detected in blood during the early stages of HIV-infection before Ab appearance).

(4) HIV enzymes: RT (**p66**), protease (**p10**), ribonuclease (**p51**) and integrase (**p31**).

• HIV is a ***highly mutable virus***. It exhibits antigenic variations not only between isolate of HIV from different places or persons but also between isolates from the same person, and even between those obtained from different sites of the same person at the same time ("*quasispecies*"). This great variability is believed to be due to the error prone nature of reverse transcription and recombination between the two genomes of HIV. Antigenic variations ("antigenic drift") is most frequent for gp120 and gp41 but also seen with other Ag.

• HIV occurs into 2 main types: **HIV-1** (high infectivity, isolated in America, Europe and central Africa) and **HIV-2** (low infectivity, isolated in West Africa). HIV-1 is related to viruses found in chimpanzees and gorillas living in western Africa, while HIV-2 viruses are related to viruses found in the endangered West African primate sooty mangabey.

• Scientists divide HIV-1 into a **major group (group M)** and two or more **minor groups** (groups O, N, P). ***HIV-1 group M*** viruses predominate and are responsible for the AIDS pandemic. Group M can be further subdivided into **subtypes** based on genetic sequence data that are also given a letter (A-K). Some of the subtypes are known to be more virulent or are resistant to different medications. *Subtype A* is common in West Africa. *Subtype B* is the dominant form in Europe, the Americas, Japan, Thailand and Australia. *Subtype C* is the dominant form in Southern Africa, Eastern Africa, India, Nepal and parts of China. *Subtype D* is generally only seen in Eastern and central Africa. *Subtype H* is limited to central Africa. *Subtype K* is limited to the Democratic Republic of Congo and Cameroon.

CULTIVATION

HIV can be cultivated by the special *co-cultivation techniques* (concurrent cultivation of patient's lymphocytes with uninfected lymphocytes in the presence of IL-2 (but it is not suitable as a routine diagnostic test).

CAUSED DISEASES

HIV-infection and **AIDS** is a disease of the human immune system caused by infection with HIV. AIDS is a condition in which progressive failure of the immune system allows development of life-threatening *opportunistic infections* and *cancers*. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype.

➢ The primary **pathogenic mechanism** of HIV-infection is damage of CD4+ T-lymphocytes. HIV infection leads to low levels of CD4+ T cells through a number of mechanisms, including *apoptosis* of uninfected bystander cells, direct viral killing of infected cells and killing of infected CD4+ T-cells by CD8 cytotoxic lymphocytes that recognize infected cells. When CD4+ T-cell numbers decline below a critical level, cell-mediated immunity is lost and the body becomes progressively more susceptible to **opportunistic infections**.

➢There are three main stages of HIV-infection: acute HIV-infection, clinical long-term latency and AIDS.

• **Acute HIV-infection** (acute retroviral syndrome) represents as influenza-like or a mononucleosis-like illness 2–4 weeks post exposure (but in 10-60% may be asymptomatic). Symptoms include fever, large tender lymph nodes, throat inflammation, rash, headache and/or sores of the mouth/genitals. Nausea, vomiting, diarrhea or peripheral neuropathy may occur. The duration of the symptoms varies, but is usually one or two weeks.

• **Clinical latency** (asymptomatic HIV or chronic HIV) without treatment, this stage of HIV-infection can last from 3 to 20 years (on average, about 8 years). While typically there are few or no symptoms at first, near the end of this stage many people experience fever, weight loss, gastrointestinal problems and muscle pain. In many cases *persistent generalized lymphadenopathy* occurs

(nonpainful enlargement of lymph nodes for over three to six months).

• **Acquired immunodeficiency syndrome** (AIDS) is defined in terms of either a CD4+ T-cell count below 200 cells per μL or the occurrence of specific diseases in association with an HIV infection. In the absence of specific treatment, around half of people infected with HIV develop AIDS within ten years. The most common initial conditions that alert to the presence of AIDS are ***pneumocystis pneumonia***, *cachexia* in the form of ***HIV-wasting syndrome*** and ***oropharyngeal candidiasis***.

Opportunistic infections may be caused by bacteria, viruses, fungi and parasites that are normally controlled by the immune system (table 39).

People with AIDS have an increased risk of developing various virus induced cancers including **Kaposi sarcoma, Burkitt's lymphoma**, **CNS lymphoma** and **cervical cancer**. Kaposi sarcoma is the most common cancer occurring in people with HIV.

Additionally, people with AIDS frequently have systemic symptoms such as prolonged fevers, sweats (particularly at night), swollen lymph nodes, chills, weakness, and weight loss. Diarrhea is another common symptom present in about 90 % of people with AIDS. They can also be affected by psychiatric and neurological symptoms (**HIV-encephalopathy**).

Table 39 — AIDS(HIV)-indicator diseases

Clinical condition	***Examples***
Bacterial infection	Pulmonary and extrapulmonary TB caused by *M.tubersulosis, M.avium* (MAC) Mycobacterioses (*M.kansasii*) Salmonellosis (septicemia) Legionellosis Listeriosis Bacterial pneumonia, recurrent Pelvic inflammatory disease (PID) caused by gonococci, chlamydia, mycoplasma/ureaplasma Campylobacteriosis Actinomycosis and nocardiosis
Viral infections	Chronic herpes simplex infection (labial, genital or anorectal, visceral) Cytomegalovirus infection (retinitis, encephalitis, colitis, pneumonia) Other herpes infections: generalized zoster, infectious mononucleosis, etc. Hairy oral leukoplakia caused by JK-virus HIV-encephalopathy (dementia)
Mycosis	Pneumocystis pneumonia Oropharyngeal candidiasis (thrush), candidiasis of trachea, bronchi, lungs Vulvovaginal candidiasis (persistent) Extrapulmonary cryptococcosis (meningitis)

	Disseminated mycosis (extrapulmonary histoplasmosis and coccidioidomycosis)
Parasitic infections	Central nervous system toxoplasmosis Chronic cryptosporidiosis (diarrhea) Chronic isosporiasis (diarrhea) Visceral leishmaniasis
Cancers	Kaposi sarcoma B-cell lymphoma Invasive cervical carcinoma Burkitt lymphoma
Other conditions	HIV-wasting syndrome Progressive multifocal leukoencephalopathy (PML) HIV-associated nephropathy or cardiomyopathy

EPIDEMIOLOGY

• Source of infection is anthroponosis. Infection occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells.

• Mechanisms of transmission are sexual contact (heterosexual and homosexual); by blood and blood products; from infected mother to babies (intrapartum/transplacental, perinatal, postnatal). Risk of transmission increases in the presence of many STDs and genital ulcers.

• HIV/AIDS is a global pandemic. As of 2012, approximately 35,3 million people have HIV worldwide with the number of new infections that year being about 2,3 million. Sub-Saharan Africa is the region most affected. In 2010, an estimated 68 % of all HIV cases and 66 % of all deaths. South and South East Asia is the second most affected region.

IMMUNITY, PREVENTION AND TREATMENT

• Currently, no cure for HIV/AIDS exists. Current **HAART** (**h**ighly **a**ctive **a**nti-**r**etroviral **t**herapy) options are combinations (or "*cocktails*") consisting of at least three medications belonging to at least two types, or "classes," of antiretroviral agents. Initially treatment is typically a *non**n**ucleoside **r**everse **t**ranscriptase **i**nhibitor* (**NNRTI**) plus two ***n**ucleoside **r**everse **t**ranscriptase **i**nhibitors* (**NRTI**). Combinations of agents which include ***p**rotease **i**nhibitors* (**PI**) are used if the above regimen loses effectiveness.

• Intensive efforts are being made to develop a vaccine and several vaccines will soon be ready for field trials (experimental vaccines include split-vaccines, DNA-vaccines, inactivated/attenuated vaccines).

• Tree principle now are propagated to prevent spreading of HIV-infection: use a good-quality condom for each sexual act; for i.v. drug consumption use only sterile syringes and needles; couples one of whom is HIV-positive should avoid unplanned pregnancy.

HERPESVIRUSES

CLASSIFICATION

Group: Group I (dsDNA)

Order: Herpesvirales
Family: Herpesviridae
Subfamily: Alphaherpesvirinae
Genus: Simplexvirus → **HSV-1** (herpes simplex virus type 1), **HSV-2** (herpes simplex virus type 2)
Genus: Varicellovirus → **VZV** (varicella zoster virus)
Subfamily: Betaherpesvirinae
Genus: Cytomegalovirus → **CMV** (cytomegalovirus)
Genus: Roseolovirus → **HHV-6** and **HHV-7** (human herpes viruses)
Subfamily: Gammaherpesvirinae
Genus: Lymphocryptovirus → **EBV** (Epstein-Barr virus)
Genus: Rhadinovirus → **HHV-8** or **KSHV** (Kaposi sarcoma herpes virus)

Alphaherpesviruses have a relatively short period of replicative cycle and tendency to cause latent infection in *sensory ganglia*. In culture they are rapidly cytopathic (cytolytic). All betahepesviruses replicate slowly. CMV replicates best in fibroblasts with a tendency to produce enlargement of infected cells (cytomegaly) and cause latent infection of salivary glands, kidneys and other organs. Other betaherpesviruses (HHV-6, HHV-7) are lymphoproliferative viruses and cause latent infection of lymphoid tissue. Gammaherpesviruses have variable cycles of reproduction and replicate in lymphoid tissue (lymphoproliferative viruses) whey they also produce latent infection.

General characteristics of all herpesviruses:
✓ Establish latent infections;
✓ Persist indefinitely in infected hosts;
✓ Frequently reactivated in immunosuppressed hosts;
✓ Some of them are cancer-causing (oncogenic).

Table 40 — Human herpesvirus classification

Type	Synonym	Sub-family	Primary target cell	Site of latency
HHV-1	**Herpes simplex virus-1** (HSV-1)	α	Mucoepithelial	Neuron
HHV-2	**Herpes simplex virus**-2 (HSV-2)	α	Mucoepithelial	Neuron
HHV-3	**Varicella zoster virus** (VZV)	α	Mucoepithelial	Neuron
HHV-4	**Epstein-Barr virus** (EBV)	γ	B-cells and epithelial cells	B-cells epithelium
HHV-5	**Cytomegalovirus** (CMV)	β	Monocyte, lymphocyte, and epithelial cells	Glands and kidneys
HHV-6 (HHV-6A, 6B)	Roseolovirus (herpes lymphotropic virus)	β	T-cells and?	T-cells and?
HHV-7	Roseolovirus (herpes lym-	β	T-cells and?	T-cells and?

	photropic virus)			
HHV-8	**Kaposi's sarcoma**-associated **herpesvirus**	γ	Lymphocyte and other cells (endothelium)	B-cells

MORPHOLOGY

Herpesviruses are **complex** and spherical (150–200 nm in diameter) with **cubic** capsid which is itself wrapped in a protein layer called the **tegument** and relatively large **double-stranded, linear DNA** genome.

ANTIGENIC STRUCTURE

Herpesviruses have no common group antigen and the different herpes viruses do not show any significant cross-reactions, except between HSV-1 and HSV-2.

CULTIVATION

Herpesviruses can be cultivated in all test-systems.

- HSV: (a) intracerebral inoculation in rabbits and mice leads to encephalitis and cornea scarification; (b) in cell cultures HSV produces different CPE (defined foci with heaped up cells, Cowdry intranuclear inclusion bodies and symplasts formation); (c) on chicken embryos CAM it produces small white shiny pocks.
- VZV can be commonly cultivated in the cell cultures (CPE is symplasts formation and intranuclear inclusions).
- CMV produces characteristic CPE in cell cultures: perinuclear cytoplasmic inclusions, intranuclear inclusions (typical of herpesviruses), symplasts are also seen. Many affected cells become greatly enlarged.

CAUSED DISEASES AND EPIDEMIOLOGY

Herpes simplex infections

- **Herpes simplex** is one of most common viral infections in human; about 60–90 % of adults are seropositive. Asymptomatic carries form the most important source of infection, especially in genital herpes. Transmission occurs by close contact, inhalation and may be sexual in genital herpes.
- **Pathogenesis**: portals of virus entry are mucous membranes and defects in the skin → multiplication of virus locally in the skin and mucous → then virus invades local nerve endings and is transported by retrograde axonal flow to sensory ganglia, where, after further replication, ***latency*** is established. Oropharyngeal HSV-1 infections result in latent infections in the *trigeminal ganglia*, whereas genital HSV-2 infections lead to latently infected *sacral ganglia*. Cell fusion provides an efficient method for cell-to-cell spread of HSV, even in the presence of neutralizing antibody. Primary HSV infections are usually mild; in fact, most are asymptomatic. Only rarely does systemic disease develop. Widespread organ involvement can result when an immunocompromised host is not able to limit viral replication and *viremia* ensues.
- **Latent infection**: virus resides in latently infected ganglia in a nonreplicating state. Viral persistence in latently infected ganglia lasts for the lifetime of

the host. Provocative stimuli can reactivate virus from the latent state, including axonal injury, fever, physical or emotional stress and exposure to ultraviolet light. The virus follows axons back to the peripheral site, and replication proceeds at the skin or mucous membranes. Spontaneous reactivations occur in spite of HSV-specific CMI and AMI in the host. However, immunity limits local viral replication, so that recurrent infections are less extensive and severe. Many recurrences are asymptomatic, reflected only by viral shedding in secretions. More than 80 % of the human population harbor HSV-1 in a latent form, but only a small portion experience recurrences. It is not known why some individuals suffer reactivations and others do not.

• **Clinical forms**: there are many clinical forms and infections may be primary or recurrent. Primary infections occur in persons without antibodies and in most individuals are clinically inapparent but result in seroconversion and establishment of latent infections in sensory ganglia. Recurrent lesions are common.

❖ ***Oropharyngeal herpes***: primary HSV-1 infection is usually *asymptomatic* (symptomatic disease occurs most frequently in small children and represents as *gingivostamatitis*). Primary infections in adults commonly cause *pharyngitis* and *tonsillitis*. Recurrent disease is characterized by a cluster of vesicles most commonly localized at the border of the lip (*labial herpes* or *"fever blisters"*). Intense pain occurs at the outset but fades over 4–5 days. Lesions are healing without scarring but may recur, repeatedly and at various intervals, in the same location. The frequency of recurrences varies widely among individuals.

❖ ***Genital herpes***: it is usually caused by HSV-2, although HSV-1 can also cause clinical episodes of genital herpes. Primary genital herpes infections can be severe, with illness lasting about 3 weeks. Genital herpes is characterized by vesiculo-ulcerative lesions of the penis of the male or of the cervix, vulva, vagina, perineum of the female. The lesions are very painful and may be associated with fever, malaise, dysuria and inguinal lymphadenopathy. Viral excretion persists for about 3 weeks. Recurrences of genital herpetic infections are common and tend to be mild. Virus is shed for only a few days. Some recurrences are asymptomatic. Whether a recurrence is symptomatic or asymptomatic, a person shedding virus can transmit the infection to sexual partners. Maternal genital herpes pose risks to both mother and fetus. Rarely, pregnant women may develop disseminated disease after primary infection, with a high mortality rate. The fetus may acquire infection as a result of viral shedding from recurrent lesions in the mother's birth canal at the time of delivery. Genital HSV infections increase acquisition of HIV-infection because the ulcerative lesions are openings in the mucosal surface.

❖ ***Other infections***: keratoconjunctivitis, skin infections, encephalitis, hepatitis, neonatal and generalized herpes.

• Because of the antigenic cross-reactivity between HSV-1 and HSV-2, preexisting immunity provides some protection against heterotypic infection. An initial HSV-2 infection in a person already immune to HSV-1 tends to be less severe.

VZV-infections

❖**Varicella (chickenpox)** is a mild, highly contagious disease, chiefly of children, characterized clinically by a generalized vesicular eruption of the skin and mucous membranes. Malaise and fever are the earliest symptoms, soon followed by the rash, first on the trunk and then on the face, the limbs, buccal and pharyngeal mucosa in the mouth.

Cases of *congenital varicella syndrome* following maternal cases of chickenpox during pregnancy have been described.

❖ **Zoster (shingles)** is a sporadic disease of adults or immunocompromised individuals that is characterized by a rash limited in distribution to the skin innervated by a single sensory ganglion. The lesions are similar to those of varicella.

• Both diseases are caused by the same virus. Varicella is the acute disease that follows primary contact with the virus, whereas zoster is the response of the partially immune host to reactivation of varicella virus present in latent form in neurons in sensory ganglia.

• The portals of virus entry are the mucosa of URT or the conjunctiva. Following initial replication in regional lymph nodes, primary viremia spreads virus and leads to replication in liver and spleen. Secondary viremia involving infected mononuclear cells transports virus to the skin, where the typical *vesicular rash* develops.

• Varicella spreads readily by airborne droplets and by direct contact. A varicella patient is probably infectious from shortly before the appearance of rash to the first few days of rash. Contact infection is less common in zoster, perhaps because the virus is absent from the upper respiratory tract in typical cases. Zoster patients can be the source of varicella in susceptible children.

• Previous infection with varicella is believed to confer lifelong immunity to varicella. Zoster occurs in the presence of neutralizing antibody to varicella.

• A live attenuated vaccine is available. Varicella in normal children is a mild disease and requires no treatment.

CMV-infections (“cytomegalic inclusion disease”)

• Cytomegaloviruses are ubiquitous herpesviruses that are common causes of human disease. CMV has an important public health problem because of its high frequency of congenital infections, which may lead to severe congenital anomalies. Severe cytomegalovirus infections are frequently found in adults who are immunosuppressed.

• CMV may be transmitted person-to-person in several different ways — direct contact, inhalation, sexual, transplacental, blood transfusion, organ transplantation. The most CMV-infections are subclinical. Like all herpesviruses, CMV establishes lifelong latent infections. Primary CMV-infections in immunosuppressed hosts (HIV patients, those receiving organ transplants, cancer patients) are much more severe than in normal hosts. Although usually less severe, reactivated infections may be as virulent as primary infections.

❖ **Clinical forms of CMV-infection in immunocompetent hosts**: prima-

ry infection is usually asymptomatic but occasionally causes a spontaneous infectious mononucleosis syndrome. CMV is estimated to cause 20–50 % of non-EBV mononucleosis cases. CMV-mononucleosis is a mild disease. Subclinical hepatitis is common.

❖ **Clinical forms of CMV-infection in immunodeficient hosts** include pneumonia, leukopenia, obliterative bronchiolitis in lung transplants, graft atherosclerosis after heart transplantation and CMV-related rejection of renal allografts. CMV often causes disseminated disease in untreated AIDS patients. Colitis/gastroenteritis and chorioretinitis are common problems, the latter often leading to progressive blindness.

❖ **Congenital infections** may result in death of the fetus *in utero*. Clinical features of congenital CMV-infection include jaundice, hepatosplenomegaly, thrombocytopenia, microcephaly, and retinitis. Mortality rates are about 20 %. The majority of survivors will develop significant CNS defects; severe hearing loss, ocular abnormalities, and mental retardation. Primary maternal infections during pregnancy are responsible for most cases of cytomegalic inclusion disease.

- There is no available vaccine.

EBV-infections

- EBV is a ubiquitous herpesvirus that is the causative agent of acute infectious mononucleosis and is associated with oral hairy leukoplakia, post-transplant lymphoproliferative syndrome (PTLD), nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkin's disease and other lymphoproliferative disorders in immunodeficient individuals.

- **EBV-antigens** are divided into three classes, based on the phase of the viral life cycle in which they are expressed:

(1) **Latent phase antigens** are synthesized by latently infected cells (**EB**V **n**uclear **a**ntigens — **EBNA 1-3** and **l**atent **m**embrane **p**roteins — **LMP 1-2**).

(2) **Early antigens** are nonstructural proteins whose synthesis is not dependent on viral DNA replication (**EBV-EA**).

(3) **Late antigens** are the structural components of the viral capsid (**v**iral **c**apsid **a**ntigen —**EBV-VCA**) and envelope (**m**embrane **a**ntigen — **EBV-MA**).

➢EBV-EA, EBV-VCA and EBV-MA indicate a *lytic infection*. LMP and EBV-encoded small RNAs (EBERs) indicate a *latent infection*. EBNAs are present at both conditions (lytic + latent).

- **Pathogenesis**: EBV is commonly transmitted by infected saliva (oral-to-oral transmission) and initiates infection in the oropharynx. Viral replication occurs in epithelial cells of the pharynx and salivary glands. It multiplies locally and invades bloodstream where infects *B-cells* (binding with their CD21 receptor). There are two types of changes in the infected B-cells: in most cases the virus becomes latent inside the lymphocytes (transformed or "*immortalized*" so they become capable of indefinite growth *in vitro*); in some cases virus produces

a lytic infection of B-cells. *Polyclonally activated* B-cells can produce many kinds of Ig (so called “heterophile Ab”). The atypical lymphocytes (“mononuclears”) are seen in blood smears during infectious mononucleosis.

Primary infections in children are usually subclinical. Reactivations of EBV latent infections can occur. These are usually clinically silent. Immunosuppression is known to reactivate infection, sometimes with serious consequences.

Clinical forms of EBV-infections:

❖ ***Infectious mononucleosis*** (“kissing disease”) is an acute self-limiting illness usually seen in nonimmune young adults following primary infection with EBV. Symptoms include fever, malaise, abnormal lymphocytes in blood and sore throat. Enlarged lymph nodes and spleen are characteristic. Some patients develop signs of hepatitis. The inapparent and manifest infections result in permanent immunity to infectious mononucleosis.

❖ ***Oral hairy leukoplakia*** is wart-like growth that develops on the tongue in some HIV-infected persons and transplant patients.

❖ **Burkitt's lymphoma** is a tumor of the jaw in African children and young adults.

❖ **Nasopharyngeal carcinoma** is cancer of epithelial cells is common in males of Chinese origin.

- There is no EBV vaccine available.

HHV-6 infections

Infections with HHV-6 typically occur in early childhood. This primary infection causes **exanthem subitum** (*roseola infantum* or *"sixth disease"*), the mild common childhood disease characterized by high fever and skin rash. The mode of transmission is presumed to be via oral secretions. Infections persist for life. Reactivation appears to be common in transplant patients. HHV-6A has been described as more neurovirulent and as such is more frequently found in patients with neuroinflammatory diseases such as *multiple sclerosis*. Also HHV-6 can cause **EBV-negative mononucleosis** and **chronic fatigue syndrome**.

HHV-7 infections

Most infections occur in childhood. Persistent infections are established in salivary glands, and the virus can be isolated from saliva. HHV-7, as well as HHV-6, can cause *roseola* and *chronic fatigue syndrome* but less frequently.

HHV-8 infections

HHV-8 has recently identified as a cofactor in induction of **Kaposi sarcoma**. It is one of significant reasons of death among AIDS-patients.

PREVENTION AND TREATMENT

- Several antiviral drugs have proved effective against HSV infections, including acyclovir, valacyclovir, etc. *Acyclovir* is currently the standard therapy. All preparations are inhibitors of viral DNA synthesis. The drugs may suppress

clinical manifestations, shorten time to healing, and reduce recurrences of genital herpes. However, HSV remains latent in sensory ganglia. Drug-resistant virus strains may emerge.

• There are inactivated varicella vaccine (for active immunization) and killed herpes simplex vaccine (for serotherapy of recurrent infections). In other case nonspecific prophylaxis is only recommended.

PAPILLOMAVIRUSES

• **Simple dsDNA** viruses with **cubic** capsid.

• Over 170 human papillomavirus (HPV) types have been completely sequenced. They have been divided into 5 genera: Alphapapillomavirus, Betapapillomavirus, Gammapapillomavirus, Mupapillomavirus and Nupapillomavirus. HPV types **16, 18**, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 are oncogenic.

• HPV establish *productive infections* only in keratinocytes of the skin or mucous membranes. Whether a malignant transformation will take place depends on cell and HPV type involved but likely on the presence of carcinogens (E5, E6, E7, etc.) as well. In carcinomas viral DNA is found in integrated form (*integrative infection*), whereas in premalignant changes (dysplasia) the viral genomes are found in the episomal state (*nonintegrative infection*).

• Most HPV infections are subclinical and will cause no physical symptoms; however, in some people subclinical infections will become clinical and may cause **benign papillomas** or **cancers** of the cervix, vulva, vagina, penis, oropharynx and anus.

• In particular, **HPV16** and **HPV18** are known to cause around 70 % of cervical cancer cases. In more developed countries, cervical screening using a Papanicolaou (Pap) test or liquid-based cytology is used to detect abnormal cells that may develop into cancer.

• All HPV infections can cause **warts** (verrucae), which are noncancerous skin growths. Types of warts include: *common skin warts* (characteristic cauliflower-like surface and are typically slightly raised above the surrounding skin); cutaneous HPV types can cause *genital* or *anal warts* (**condylomas**) but are not associated with the development of cancer; *plantar warts* are found on the soles of the feet (causing pain when walking); warts form under the fingernail (*subungual*), around the fingernail or on the cuticle (*periungual*); *flat warts*.

• Other infections: cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), penile intraepithelial neoplasia (PIN), and/or anal intraepithelial neoplasia (AIN); respiratory papillomatosis, in which warts form on the larynx or other areas of the respiratory tract.

• Source of infection is humans. Mechanisms of transmission are direct contact (sexual contact for condylomas) and autoinfection (for warts).

• Two vaccines are available to prevent infection by some HPV types: *Gardasil* and *Cervarix*. Both protect against initial infection with HPV types 16

and 18, which cause most of the HPV associated cancer cases.

POLYOMAVIRUSES

- **Siple dsDNA** viruses with **cubic** capsid.
- They are potentially oncogenic; they often persist as latent infections in a host without causing disease, but may produce tumors in a host of a different species, or a host with an ineffective immune system. The name *polyoma* refers to the viruses ability to produce multiple (poly-) tumors (-oma).
- The polyomaviruses can be divided into two groups: in one group are SV-40 (simian vacuolating virus 40) and SV-4-like viruses such as human pathogen JC virus and BK virus (the initials of the first patients in whom these viral types were identified). In other are the true polyomaviruses such as *oncogenic zoopathic viruses*.
- **JC virus** can infect the respiratory system, kidneys, or brain (sometimes causing the fatal ***progressive multifocal leukoencephalopathy*** — PML). PML is fatal demyelinating disease of the drain ("slow viral infection") which has become more frequent as a sequel to HIV-infection but is otherwise rare.
- **BK virus** produces a mild respiratory infection, can affect the kidneys of immunosuppressed transplant patients and causes ***hemorrhagic cystitis*** in bone marrow transplantation patients.
- The JC and BK viruses are widespread (80 % of human population show Ab to them), despite which, clinical manifestations are rare and present during suppression of immune system. Transmission routes have not been clarified.

PARVOVIRUSES

- Parvoviruses are smallest (20 nm), **simple** viruses with **cubic** capsid and linear, **non-segmented single-stranded DNA** genome.
- Some parvoviruses can only replicate in the presence of a helper virus (adenovirus or herpesvirus). Parvovirus B19, the human pathogenic virus, is capable of autonomic replication.
- **Parvovirus B19** replicates in the bone marrow in erythrocyte precursor cells which are destroyed in the process. In patient already suffering from anemia such infection result in so-called ***aplastic crises*** (lack of erythrocyte resupply leads to a critical shortage). In otherwise healthy persons usually have asymptomatic infection.
- They also can cause harmless epidemic infection in children, ***erythema infectiosum*** ("slapped-cheek syndrome" or fifth disease) which is characterized by sudden onset of exanthem on the face and extremities. Certain forms of ***arthritis*** are considered as complications of B19 infection.
- This virus also appears to cause ***spontaneous abortions*** in early pregnancy and fatal damage in late pregnancy (***hydrops fetalis***).
- The transmission mechanism of parvoviruses is not known (inhalation

and fecalo-oral routes are suspected, blood is also infectious).

• Human **bocaviruses** were first isolated in 2005 in Sweden. They may be able to cause hepatitis in an immunosuppressed host and AVRI in children.

Lecture 21. MYCOLOGY. PATHOGENIC FUNGI

Mycology is the study of fungi. Approximately 80 000 species of fungi have been described, but fewer than 400 are medically important, and less than 50 species cause more than 90 % of **mycoses** of humans and animals. Rather, most species of fungi are beneficial to humankind. They reside in nature and are essential in breaking down and recycling organic matter. Some fungi greatly enhance our quality of life by contributing to the production of food and spirits, including cheese, bread and beer. Other fungi have served medicine by providing useful bioactive secondary metabolites such as antibiotics (eg, penicillin) and immunosuppressive drugs (eg, cyclosporine).

The mycoses with the highest incidence (candidiasis and dermatophytosis) are caused by fungi that are part of the normal microbial flora or highly adapted to survival on the human host. Most patients who develop opportunistic infections have serious underlying diseases and compromised host defenses. During infection, most patients develop significant cellular and humoral immune responses to the fungal antigens.

Pathogenic fungi do not produce potent toxins and the mechanisms of fungal pathogenicity are complex. Most mycoses are difficult to treat.

GENERAL CHARACTERISTCS OF FUNGI

• All fungi are eukaryotic organisms. Each fungal cell has at least one nucleus and nuclear membrane, endoplasmic reticulum, mitochondria and secretory apparatus.

• Most fungi are obligate or facultative aerobes. They are chemotrophic, secreting enzymes that degrade a wide variety of organic substrates into soluble nutrients which are then passively absorbed or taken into the cell by active transport.

• Saprophytes or parasites. They do not cause widespread epidemics. Most

pathogenic fungi are exogenous, their natural habitats being water, soil and organic debris.

• Fungi grow in two basic forms, as **yeasts** and **molds (**or **moulds).** Growth in the mold form occurs by production of multicellular filamentous colonies. These colonies consist of branching cylindric tubules called **hyphae**. The mass of intertwined hyphae that accumulates during active growth is a **mycelium.** Some hyphae are divided into cells by cross-walls or **septa**, typically forming at regular intervals during hyphal growth. Hyphae that penetrate the supporting medium and absorb nutrients are the vegetative or *substrate hyphae*. In contrast, *aerial hyphae* project above the surface of the mycelium and usually bear the reproductive structures (exospores) of the mold.

• **Yeasts** are single cells, usually spherical to ellipsoid in shape. Most yeasts reproduce by *budding*. Some species produce buds that characteristically fail to detach and become elongated; continuation of the budding process then produces a chain of elongated yeast cells called **pseudohyphae**.

• Most fungi occur in nature and grow readily on simple sources of nitrogen and carbohydrate. The traditional mycological medium, **Sabouraud's agar**, which contains glucose and modified peptone (pH 7,0), has been used because it does not readily support the growth of bacteria. To culture medical fungi from nonsterile specimens, antibacterial antibiotics (eg, gentamicin, chloramphenicol) and cycloheximide are added to the media to inhibit bacteria and saprophytic molds, respectively.

• Molds produce **R-colonies** with characteristic features such as rates of growth, texture and pigmentation. Yeast **S-colonies** are usually soft, opaque, 1–3 mm in size and cream-colored.

• Some species of fungi are **dimorphic** and capable of growth as a yeast or mold depending on environmental conditions (*saprophytic phase is mycelium, parasitic phase is budding cells*).

• All fungi have an essential rigid cell wall that determines their shape. Cell walls are composed largely of carbohydrates polysaccharides as well as glycoproteins and lipids. During infection, fungal cell walls have important pathogenic properties. The surface components of the cell wall mediate attachment of the fungus to host cells. Cell wall polysaccharides may activate the complement cascade and provoke an inflammatory reaction. Cell walls release antigens that may elicit cellular immune response and host antibodies.

• In addition to their vegetative growth as yeasts or molds, fungi can produce spores to enhance their survival. Spores can derive from asexual or sexual reproduction. Asexual spores are genetically identical. The medical fungi produce two major types of asexual spores, **conidia** and, in the zygomycetes, **sporangiospores**. Informative feature of spores includes their morphology (size, shape, texture, color, and unicellularity or multicellularity). In some fungi, vegetative cells may transform into conidia (eg, arthroconidia, chlamydospores). In

others, conidia are produced by a conidiogenous cell, which itself may be attached to a specialized hypha called a conidiophore.

• Antifungal drugs (antimycotics) are used to treat mycoses. Depending on the nature of the infection, a topical or systemic agent may be used. Example of antifungals include: ***fluconazole*** which is the basis of many over-the-counter antifungal treatments. Another example is ***amphotericin B*** which is more potent and used in the treatment of the most severe fungal infections that show resistance to other forms of treatment and it is administered intravenously. Yeast infections in the vagina, caused by *Candida albicans*, can be treated with medicated suppositories such as tioconazole and pessaries whereas skin yeast infections are treated with medicated ointments.

• Prevention is only nonspecific. There is no available vaccine. Keeping the skin clean and dry, as well as maintaining good hygiene will help to avid topical mycoses. Because fungal infections are contagious, it is important to wash after touching other people or animals. Sports clothing should also be washed after use.

CLASSIFICATION OF FUNGI

The fungi are classified in four phyla: Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota. The largest phylum is the Ascomycota (or **ascomycetes**), which includes more than 60 % of the known fungi and about 85 % of the human pathogens. The remaining pathogenic fungi are **zygomycetes** or **basidiomycetes**. A fungal species is assigned to a phylum, as well as the appropriate Class, Order, and Family, based on its mode of sexual reproduction, morphology, physiology, and phylogenetic relationships.

CLASSIFICATION OF MYCOSES

1. Superficial mycosis
2. Cutaneous mycosis
3. Subcutaneous mycosis
4. Systemic (deep) mycosis
5. Opportunistic mycosis
6. Mycotoxicosis (food intoxication)

Superficial mycoses

❖ **Tinea nigra** is a superficial chronic and asymptomatic infection of the stratum corneum. This condition is more prevalent in warm coastal regions and among young women. The lesions appear as a dark (brown to black) discoloration, often on the palm. Microscopic examination of skin scrapings from the periphery of the lesion will reveal branched, septate hyphae and budding yeast cells with melaninized cell walls. Tinea nigra will respond to treatment with keratolytic solutions, salicylic acid, or azole antifungal drugs.

❖ **Black piedra** is a nodular infection of the hair shaft. **White piedra** presents as larger, softer, yellowish nodules on the hairs. Axillary, pubic, beard, and scalp hair may be infected. Piedra is endemic in tropical underdeveloped countries.

Cutaneous mycoses

Cutaneous mycoses are caused by fungi that infect only the superficial keratinized tissue (skin, hair, and nails). The most important of these are the **Dermatophytes** (Microsporum, Trichophyton, and Epidermophyton).

Dermatophytoses are among the most prevalent infections in the world. Although they can be persistent and troublesome, they are not debilitating or life-threatening. In skin they are diagnosed by the presence of hyaline, septate, branching hyphae or chains of arthroconidia. In culture, the many species are closely related and often difficult to identify.

Dermatophytes are classified as geophilic, zoophilic, or anthropophilic depending on whether their usual habitat is soil, animals, or humans. Several dermatophytes that normally reside in soil or are associated with particular animal species are still able to cause human infections. Anthropophilic species, which cause the greatest number of human infections, cause relatively mild and chronic infections in humans, produce few conidia in culture, and may be difficult to eradicate. Conversely, geophilic and zoophilic dermatophytes, being less adapted to human hosts, produce more acute inflammatory infections that tend to resolve more quickly. Dermatophytes are acquired by contact with contaminated soil or with infected animals or humans.

Dermatophyte infections begin in the skin after trauma and contact. There is evidence that host susceptibility may be enhanced by moisture, warmth, specific skin chemistry, composition of sebum and perspiration, youth, heavy exposure, and genetic predisposition. The incidence is higher in hot, humid climates and under crowded living conditions. Wearing shoes provides warmth and moisture, a setting for infections of the feet. The source of infection is soil or an infected animal in the case of geophilic and zoophilic dermatophytes, respectively. The conidia can remain viable for long periods. Anthropophilic species may be transmitted by direct contact or through fomites, such as contaminated towels, clothing, shared shower stalls, and similar examples.

Dermatophyte infections were mistakenly termed ringworm or tinea because of the raised circular lesions. The clinical forms are based on the site of involvement. A single species is able to cause more than one type of clinical infection. Conversely, a single clinical form, such as *tinea corporis*, may be caused by more than one dermatophyte species. Very rarely, immunocompromised patients may develop systemic infection by a dermatophyte.

❖ **Tinea Pedis** (*Athlete's Foot*) is the most prevalent of all dermatophytoses. It usually occurs as a chronic infection of the toe webs. Other varieties are the vesicular, ulcerative, and moccasin types, with hyperkeratosis of the sole. Initially, there is itching between the toes and the development of small vesicles that rupture and discharge a thin fluid. The skin of the toe webs becomes macerated and peels, whereupon cracks appear that are prone to develop secondary bacterial infection.

❖ **Tinea capitis** is dermatophytosis or ringworm of the scalp and hair. The

infection begins with hyphal invasion of the skin of the scalp, with subsequent spread down the keratinized wall of the hair follicle. Infection of the hair takes place just above the hair root. The hyphae grow downward on the nonliving portion of the hair and at the same rate as the hair grows upward. The infection produces dull gray, circular patches of alopecia, scaling and itching.

❖ **Candidiasis of skin, mucosa** or **nails.**

Subcutaneous mycoses

The fungi that cause subcutaneous mycoses normally reside in soil or on vegetation. Subcutaneous mycoses involve the dermis, subcutaneous tissues, muscle and fascia. These infections are chronic and can be initiated by piercing trauma to the skin which allows the fungi to enter. These infections are difficult to treat and may require surgical interventions such as debridement. In general, the lesions become granulomatous and expand slowly from the area of implantation. Extension via the lymphatic draining the lesion is slow except in sporotrichosis. These mycoses are usually confined to the subcutaneous tissues, but in rare cases they become systemic and produce life-threatening disease.

❖ **Chromoblastomycosis** occurs most commonly in tropical or subtropical climates, often in rural areas. It can be caused by many types of fungi which become implanted under the skin, often by thorns or splinters. Chromoblastomycosis spreads very slowly; it is rarely fatal and usually has a good prognosis, but it can be very difficult to cure.

❖ **Sporotrichosis** usually affects the skin, although other rare forms can affect the lungs, joints, bones, and even the brain. Because roses can spread the disease, it is one of a few diseases referred to as rose-thorn or rose-gardeners' disease. It enters through small cuts and abrasions in the skin to cause the infection. In case of sporotrichosis affecting the lungs, the fungal spores enter through the respiratory pathways. Sporotrichosis can also be acquired from handling cats with the disease; it is an occupational hazard for veterinarians.

Systemic mycoses

Systemic mycoses due to primary pathogens originate primarily in the lungs and may spread to many organ systems. Organisms that cause systemic mycoses are inherently virulent. In general primary pathogens that cause systemic mycoses are dimorphic.

❖ **Coccidioidomycosis** is a mammalian fungal disease caused by *Coccidioides immitis* or *Coccidioides posadasii*. It is endemic in certain parts of Arizona, California, Nevada, and northern Mexico. Infection is caused by inhalation of the particles. The disease is not transmitted from person to person. Symptomatic infection usually presents as an influenza-like illness with fever, cough, headaches, rash, muscle pain and joint pain. Some patients fail to recover and develop chronic pulmonary infection or widespread disseminated infection (affecting meninges, soft tissues, joints and bone). Severe pulmonary disease may develop in HIV-infected persons.

❖ **Histoplasmosis** is a disease caused by the fungus *Histoplasma capsulatum*. Symptoms of this infection vary greatly, but the disease affects primarily the lungs. Occasionally, other organs are affected; this is called disseminated histoplasmosis, and it can be fatal if left untreated. Histoplasmosis is common among AIDS patients because of their suppressed immunity. *Histoplasma capsulatum* is found in soil, often associated with decaying bat guano or bird droppings. Disruption of soil from excavation or construction can release infectious elements that are inhaled and settle into the lung. It is endemic in certain areas of USA. It is also common in caves in southern and East Africa.

❖ **Blastomycosis** is a fungal infection of humans and other animals, notably dogs and occasionally cats, caused by the organism *Blastomyces dermatitidis*. endemic to portions of North America, blastomycosis causes clinical symptoms similar to histoplasmosis.

Systemic opportunistic mycoses

Systemic mycoses due to opportunistic pathogens are infections of patients with immune deficiencies who would otherwise not be infected. Examples of immunocompromised conditions include AIDS, alteration of normal flora by antibiotics, immunosuppressive therapy and metastatic cancer.

❖ **Aspergillosis** is the name given to a wide variety of diseases caused by infection by fungi of the genus Aspergillus. The majority of cases occur in people with underlying illnesses such as tuberculosis or chronic obstructive pulmonary disease, but with otherwise healthy immune systems. Most commonly, aspergillosis occurs in the form of chronic pulmonary aspergillosis, aspergilloma or allergic bronchopulmonary aspergillosis. Other, noninvasive manifestations include fungal sinusitis, otomycosis (ear infection), keratitis (eye infection) and onychomycosis (nail infection). In most instances these are less severe, and curable with effective antifungal treatment.

Acute invasive aspergillosis occurs when the immune system fails to prevent Aspergillus spores from entering the bloodstream via the lungs. The most frequently identified pathogen is *Aspergillus fumigatus*. It is estimated that most humans inhale thousands of Aspergillus spores daily, but they do not impact on most health persons due to effective immune responses.

❖ **Cryptococcosis** is a potentially fatal fungal disease. It is caused by one of two species; *Cryptococcus neoformans* and *Cryptococcus gattii*. Cryptococcosis is believed to be acquired by inhalation of the infectious propagule from the environment. Cryptococcosis is a defining opportunistic infection for AIDS, and is the second-most-common AIDS-defining illness in Africa. Other conditions that pose an increased risk include certain lymphomas, sarcoidosis, liver cirrhosis and patients on long-term corticosteroid therapy. Distribution is worldwide in soil. In humans, *C. neoformans* causes three types of infections: wound or cutaneous cryptococcosis, pulmonary cryptococcosis, cryptococcal meningitis.

❖ **Systemic candidiasis.**

Candidiasis is a mycosis of any species from the genus Candida. *Candida albicans* is the most common agent of candidiasis in humans.

Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases. There are many clinical forms of candidiasis: *oral candidiasis* (thrush, oropharyngeal candidiasis), *candidal vulvovaginitis* (vaginal yeast infection), *candidal balanitis*, infection of the glans penis, almost exclusively occurring in uncircumcised male, *cutaneous candidiasis* (ex.: chronic mucocutaneous candidiasis), *onychomycosis* (nail infection), *systemic candidiasis* (one organ system involved), *disseminated candidiasis* (multiple system involvement, which sometimes follows "candidemia", a form of fungemia), *antibiotic candidiasis* (iatrogenic candidiasis).

Symptoms of candidiasis vary depending on the area affected. Most candidial infections result in minimal complications such as redness, itching, and discomfort, though complications may be severe or even fatal if left untreated in certain populations. In immunocompetent persons, candidiasis is usually localized infection of the skin or mucosal membranes. Infection of the vagina or vulva may cause severe itching, burning, soreness, irritation and a whitish (whitish-gray) cottage cheese-like discharge. These symptoms are also present in the more common bacterial vaginosis.

Candida yeasts are generally present in healthy humans, particularly on the skin (also in oral cavity and colon), but their growth is normally limited by the human immune system and by competition with normal flora (also in the case of skin, by the relative dryness of the skin, as Candida requires moisture for growth). *C.albicans* was isolated from the vaginas of 19 % of apparently healthy women. External use of detergents or douches or internal disturbances (hormonal or physiological) can perturb the normal vaginal flora, consisting of lactobacilli, and result in an overgrowth of Candida cells, causing symptoms of infection, such as local inflammation. Pregnancy and the use of oral contraceptives have been reported as risk factors. Diets high in simple carbohydrates have been found to affect rates of oral candidiasis. Wearing wet swimwear for long periods of time is also believed to be a risk factor.

Diseases or conditions linked to candidiasis include HIV/AIDS, mononucleosis, cancer treatments, steroids, stress, and nutrient deficiency. In penile candidiasis, the causes include sexual intercourse with an infected individual, low immunity, antibiotics and diabetes. Male genital yeast infections are less common and incidences of infection are only a fraction of those in women; however, yeast infection on the penis from direct contact via sexual intercourse with an infected partner is not uncommon.

Candidiasis is commonly treated with antimycotics; these antifungal

drugs include topical clotrimazole, topical nystatin, fluconazole and topical ketoconazole.

Lecture 22. INTRODUCTION INTO CLINICAL MICROBIOLOGY

Clinical microbiologists determine the nature of infectious disease and test the ability of various antibiotics to inhibit or kill the isolated microorganisms. In addition to bacteriology, a contemporary clinical microbiologist is responsible for a wide range of microscopic and cultural studies in mycology, parasitology, and virology. The clinical microbiologist is often the most competent person available to determine the nature and extent of hospital-acquired infections, as well as public-health problems that affect both the hospital and the community.

HOSPITAL INFECTIONS

Hospitals have always acted as a source of infection to patients admitted to them. The concept of asepsis and its application in hospital practice reduces their incidence, but these infections still cause considerable morbidity and mortality. Even when hospitalization does not lead to obvious infection, it causes a change of the patient's normal flora which being gradually replaced by the drug resistant microorganisms typical of the hospital environment (**endogenous infection**).

- The terms **hospital** (intrahospital) infection, **hospital-acquired infection** (**HAI**) or **nosocomial** infection are applied to infections developing in hospitalized patients, not present or in incubation at the time of their admission. Such infections stay become evident during their stay in hospital, or, sometimes, only after their discharge.
- HAI are typically **exogenous**, the source being any part of the hospital ecosystem, including people, objects, food, water and air in the hospital.
- Such infections may be **iatrogenic** in that may be induced by some diagnostic or therapeutic intervention in the hospital.

• They may be **opportunistic** in that microorganisms of low virulence may cause disease in hospitalized patients whose immune system is impaired.

• Hospital infections may occur sporadically or as outbreaks.

• Several factors contribute to the occurrence and severity of hospital infections: (1) many patients in hospitals have impaired defense mechanisms due to their main disease or administered therapy (antibiotics, corticosteroids, etc.). (2) Hospital environment is heavily laden with a wide variety of pathogens; patients and medical stuff shed them from their hands, clothes, etc. Bedding, linen and utensils act as fomites. Equipment may be contaminated. Pathogens are present in the hospital dust and air, and sometimes even in antiseptics lotions and ointments. Contamination of hospital food and water can cause outbreaks of infections. (3) Major invasive procedures, diagnostic or therapeutic interventions are carried out only in hospitals; the slightest lapse in asepsis during these actions can lead to infection. (4) HAIs are generally more serious and refractory to treatment as the infectious agents are resistant to antibiotics. (5) Hospital infections are in a sense diseases of medical progress. Advances in treatment of cancer, organ transplantation, implanted prostheses and other sophisticated medical technologies enhance the risk of infection to patients.

MICROBIOLOGY OF HOSPITAL INFECTIONS

Almost any pathogen can, on occasion, cause hospital infection but those that are able to survive in the hospital environment for long periods and develop resistance to antibiotics and disinfectants are particularly important in this respect.

• *Staphylococcus aureus* (and sometimes *S.epidermidis*) are "hospital staphylococci" are common agents of HAIs. Group D streptococci (enterococci) also are responsible for many hospital infections.

• Enteric Gram-negative bacilli (*E.coli*, Klebsiella, Enterobacter, Proteus, Serratia, etc.) have become the most important group of hospital pathogens. Resistant Salmonella strains (especially *S.Typhimurium*) became a prominent hospital pathogen.

• *Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia* (and other Gram-negative nonfermentaing rods) have always been important causes of hospital infections because of their high resistance to most antibiotics and ability to survive and even multiply at low temperatures and in disinfectants (antiseptic) solutions.

• *Clostridium difficile* colitis or pseudomembranous colitis is colitis resulting from infection with *Clostridium difficile*, a type of spore-forming bacteria. The colitis is thought to occur when this bacteria replaces normal gut flora that has been compromised, usually following antibiotic treatment for an unrelated infection ("antibiotic-associated diarrhea").

• HIV, hepatitis B and C are the important infection transmitted through blood and blood products. Screening of donors has checked this risk to a large extent.

• Cytomegalovirus, influenza virus, RSV, enteroviruses and adenoviruses may also cause hospital infections.

• The range of hospital pathogens also includes yeasts (*Candida albicans*), molds (Aspergillus, Mucor) and some protozoa (*Entamoeba histolitica*).

COMMON TYPES OF HOSPITAL INFECTIONS

❖ **Wound infection**: Several factors influence the occurrence of post-operative wound infections (delayed wound healing or abscess), such as the site and duration of surgery, immune status of patient and skill of the operator. Most important causative agents are Staphylococci, Streptococci, Clostridia, Gram-negative rods, etc. Nonsurgical sites of wound infections include infection "cut-downs", umbilical stumps, ulcers and burns. *P.aeruginosa* is the most important cause in burns. Tetanus as a result of HAI is now rare but should be kept in mind and toxoid administered to nonimmune patients before elective surgery. Many cases of neonatal tetanus in developing countries due to the use of contaminated umbilical cord ties.

❖ **Urinary tract infections (UTIs)**: Causative agents are *E.coli*, Proteus, *P.aeruginosa* and other Gram-negative rods. Mixed infection is common. Infection can be prevented by strict asepsis during catheterization. Indwelling catheters are to be used only when unavoidable, and then only with proper closed drainage. UTI represents as simple *cystitis* (bladder infection) or as *pyelonephritis* (kidney infection). Urine is normally a sterile bodily fluid when inside the bladder, but can pick up commensals and pathogens when exiting through the urethra. *Bacteriuria* (bacteria in the urine), especially Gram-negative rods, usually indicate UTI. *Asymptomatic bacteriuria* is bacteriuria without accompanying symptoms of a urinary tract infection (such as frequent urination, painful urination or fever). It is more common in women, in the elderly, in residents of long-term care facilities, and in patients with bladder catheters.

❖ **Respiratory infections**: Aspiration in unconscious patients and pulmonary ventilation (ventilator-associated pneumonia — VAP) or instrumentation may lead to nosocomial *pneumonia,* particularly in those with pre-existing cardiopulmonary disease. MRSA and Gram-negative rods (*P.aeruginosa*, Enterobacter, Klebsiella, etc.) are the common pathogens (rare viruses — influenza virus, RSV and, in immunocompromised persons, CMV). Antibiotic treatment is unsatisfactory. Postural drainage is useful in the prevention and management in such cases. *Hospital-acquired pneumonia* (HAP) or nosocomial pneumonia refers to any pneumonia contracted by a patient in a hospital at least 48–72 hours after being admitted. It is thus distinguished from community-acquired pneumonia.

❖ **Bacteremia** and **septicemia**. These may be consequences of infections at any site but are commonly caused by infected intravenous cannula (thin needle inserted into a vein or body cavity to administer medicine, drain off fluid, or insert a surgical instrument). The longer the cannula kept in situ, the greater risk of infection. "Cut-downs" (procedure of cutting into a vein in order to insert a needle or cannula) on the leg veins in infants or children with diarrhea generally get left in place for long periods, the site being bathed in diarrheal stools. Phlebitis sets in with consequent bacteremia. Many of children admitted with di-

arrhea thus die of septicemia. Gram-negative rods are the common pathogens. Infection can be prevented by proper skin antiseptics before medical manipulations. *S.epidermidis* bacteremia is seen commonly inpatients with artificial heart valves. Bacteremia in those with valvular defects may lead to *endocarditis*.

DIAGNOSIS OF HOSPITAL INFECTIONS

- Etiological diagnosis is by the routine microbiological methods such as bacteriological (cultivation) and bacterioscopical.
- When an outbreak occurs, the source of infection should be identified. This requires the sampling of possible sources of infection such as hospital staff, patients, inanimate objects, water, air or food. Typing of isolates – phage typing, bacteriocin typing, antibioticogram (sensitivity tests) or biotyping – from cases and sites may indicate a causal connection.
- Obvious examples of sources of hospital outbreaks are nasal carriers of staphylococci by surgeons or Pseudomonas growth in hand lotions. Carriers should be suitable treated.

PREVENTION OF HOSPITAL INFECTIONS

The measures taken to control and prevent nosocomial infections correspond in the wider sense to the general methods of **infection control**. Every major hospital must have "*infections control teams*' consisting of microbiologists, medical and nursing staff and hospital administrators. Besides investigating and controlling outbreaks, their functions include formulation appropriates guidelines for admission, nursing and treatment of infectious patients, asepsis practices, determining antibiotic policies and immunization schedules, education of patients and hospital personnel on infection control. Such measures help in reduction the incidence of hospital infections, even if they do not eliminate them altogether.

- Hospitals have sanitation protocols regarding uniforms, equipment sterilization, washing, and other preventive measures.
- Operation measures include asepsis, disinfection, sterilization and cleaning. Further precautionary operational measures include isolation of patients that would be sources of infection and the economical and specific administration of antibiotics.
- The organization of hospital infection control must be adapted to the structure of each particular hospital with help of *hospital epidemiologists*.
- Thorough hand washing and/or use of alcohol rubs by all medical personnel before and after each patient contact is one of the most effective ways to combat nosocomial infections. In addition to hand washing, gloves play an important role in reducing the risks of transmission of microorganisms. Wearing an apron during patient care reduces the risk of infection.
- Touch surfaces commonly found in hospital rooms, such as bed rails, call buttons, touch plates, chairs, door handles, light switches, grab rails, intravenous poles, dispensers (alcohol gel, paper towel, soap), dressing trolleys, and counter and table tops are known to be contaminated with Staphylococcus, MRSA and VRE.

• More careful use of antimicrobial agents, such as antibiotics, is also considered vital. Unfortunately, in many hospitals, in many hospitals, infection control is attempted by resorting to more and more of antibiotics. This is not only futile but may even be positively harmful by encouraging selective colonization by multiresistant pathogens. While antibiotic drugs to treat diseases caused by Gram-positive MRSA are available, few effective drugs are available for *Acinetobacter*. *Acinetobacter* bacteria are evolving and becoming immune to existing antibiotics. Another growing disease is the drug-resistant, Gram-negative *Klebsiella pneumoniae* (resistant to virtually all modern antibiotics, and those supergerms are now spreading worldwide). "*For Gram-positives we need better drugs; for Gram-negatives we need any drugs*," said medical specialists (Dr. Brad, author of *Rising Plague*, a book about drug-resistant pathogens).

Учебное издание

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**МИКРОБИОЛОГИЯ,
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(на английском языке)**

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для студентов 2 и 3 курсов факультета
по подготовке специалистов для зарубежных стран медицинских вузов**

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