**MEDICALMICROBIOLOGY**

**Brief history of microbiology**

**• Robert Hook (1665)** – reported that life’s smallest structural units were ‘little boxes’ or ‘cells’. This marked the beginning of cell theory – that all living things are composed of cells.

**• Van Leuwenhoek (1673)** – discovered the ‘invisible’ world of microorganisms’ ‘animalcules.

Until second half of nineteenth century many believed that some forms of life could arise spontaneously from non-living matter – spontaneous generation.

**• Francesco Redi (1668)** – Strong opponent of spontaneous generation. He demonstrated that maggots appear on decaying meat only when flies are able to lay eggs on the meat.

**• John Needham (1745)** – claimed that microorganisms could arise spontaneously from heated nutrient broth.

**• Lazzaro Spallanzani (1765)** – repeated Needhams experiments and suggested that Needham’s results were due to microorganisms in the air entering the broth.

**• Rudolf Virchow (1858)** – concept of biogenesis – living cells can arise only from preexisting cells.

**• Louis Pasteur (1822-1895)** – Pasteur’s experiments on swan shaped necks resolved the controversy of spontaneous generation. His discoveries led to the development of aseptic techniques used in the laboratory and medical procedure to prevent contamination by microorganisms that are in the air.

**Golden age of microbiology:**

• Rapid advances in the science of microbiology were made between 1857 and 1914. This included Fermentation and Pasteurization:

• Pasteur found that yeast ferments sugars to alcohols and that bacterium can oxidize the alcohol to acetic acid.

• Heating processes called pasteurization is used to kill bacteria in some alcoholic beverages and milk.

**The Germ theory of disease:**

**• Agostino Bassi (1934) and Pasteur (1865)** – showed a causal relationship between microorganisms and disease.

**• Joseph Lister (1860s)** – introduced the use of disinfectant to clean surgical dressings in order to control infection in humans

**• Robert Koch (1876)** – proved that microorganisms transmit disease – Koch’s postulates are still used today to prove that a particular microorganism causes a particular disease. He Introduced pure cultures.

**Koch’s postulates (Henle-Koch’s Postulates) are**

1. A specific organism should be found constantly in association with the disease.

2. The organism should be isolated and grown in a pure culture in the laboratory.

3. The pure culture when inoculated into a healthy susceptible animal should produce symptoms/lesions of the same disease

4. From the inoculated animal, the microorganism should be isolated in pure culture.

5. An additional criterion introduced is that specific antibodies to the causative organism shouldbe demonstrable in patient’s serum.

Koch also developed techniques for isolating organisms. Identified the bacillus that causes tuberculosis and anthrax, developed tuberculin and studied various diseases in Africa and Asia. His studies on Tuberculosis won him Nobel Prize for philosophy and medicine in 1905.

**Vaccination**:

• Immunity is conferred by inoculation with a vaccine.

**• Edward Jenner (1798)** – demonstrated that inoculations with cowpox material provides humans with immunity from small pox

• Pasteur (1880) – discovered that avirulent bacteria could be used as a vaccine for chicken cholera; he coined the word vaccine

• Modern vaccines are prepared from living avirulent microorganisms or killed pathogens, from isolated components of pathogens, and by recombinant DNA techniques.

**Emergence of special fields of Microbiology:**

**Immunology:**

• Immunization was first used against small pox. Edward Jenner used fluid from cowpox blisters to immunize against it.

• **Pasteur** developed techniques to weaken organisms so they would produce immunity without producing disease.

• **Elie Metchnikoff** discovered that certain cells in the body would ingest microbes and named them as phagocytes.

**Industrial Microbiology and Microbial ecology**:

• Pasteur – fermentation technology and pasteurization. One of his most important discoveries was that some fermentative microorganisms were anaerobic and others were able to live either aerobically or anaerobically.

**Microbial ecology – Two pioneers** –

• **Sergei N. Winogradsky (1856-1953**) – Soil microbiology – discovered that soil bacteria could oxidize iron, sulfur and ammonia to obtain energy and many bacteria incorporate CO2 into organic matter. He also isolated anaerobic nitrogen fixing soil bacteria and studied the decomposition of cellulose.

**• Martinus Beijerinck (1851-1931**) – He isolated aerobic nitrogen fixing bacterium Azotobacter, a root nodule bacterium also capable of fixing nitrogen (later renamed as Rhizobium); and sulfate reducing bacteria. Both of them developed enrichment culture technique and use of selective media, which have been of great importance in microbiology.

**Virology:**

**• Beijerinck** characterized viruses as pathogenic molecules that could take over a host cells mechanism for their own use

• **Wendell Stanley (1935**) – crystallized TMV and crystals consisted of protein and RNA. • Viruses were first observed with an EM in 1939. •

**Alfred Hershey and** **Martha Chase (1952) –** demonstrated that the genetic material of some viruses is DNA

**• James Watson and Francis Crick (1953)** -determined the structure of DNA

**Chemotherapy:**

• There are two types of chemotherapeutic agents: synthetic drugs and antibiotics.

**• Elrlich (1910)** introduced an arsenic containing chemical called Salvarsan to treat Syphilis. • **Alexander Fleming (1928)** – observed that the mold Penicillium inhibited the growth of bacteria and named the active ingredient as penicillin. Penicillin has been used clinically as an antibiotic since the 1940s. Domagk and others developed sulfa drugs. • Waksman and others developed Streptomycin and other antibiotics derived from soil organisms. • Researchers are tackling the problem of drug-resistant microbes.

**Genetics and Molecular Biology:**

• 1900 – Modern genetics began with the rediscovery of Gregor Mendel's principles of genetics. • **Frederick Griffith (1928) -** discovered that previously harmless bacteria could change their nature and become capable of causing disease

**• Avery, McCarty and MacLeod (1940’s)** – showed that this genetic change was due to DNA. After this finding came the crucial discovery of the structure of DNA by Watson and Crick • **Edward Tatum and George Beadle** – studied biochemical mutants of Neurospora to show how genetic information controls metabolism.

**• Barbara McClintock (1950)** – discovered that some genes could move from one location to another on a chromosome. • Early 1960’s witnessed a further explosion of discoveries relating to the way DNA controls protein synthesis.

**• Francois Jacob and Jacques Monod (1961)** – discovered mRNA and later made the first major discoveries about regulation of gene function in bacteria. • Microorganisms can now be genetically engineered to manufacture large amounts of human hormones and other urgently needed medical substances.

**• Late 1960’s Paul Berg** showed that fragments of human or animal DNA that code for important proteins can be attached to bacterial DNA. The resulting hybrid was the first example of recombinant DNA.

**The future Micro biology**

Microbiology has been in the forefront of research in medicine and biology and continues to play a role in Genetic engineering and Gene therapy.

Genetic engineering – scientists are attempting to redesign microorganisms for a variety of purposes (drugs, hormones, vaccines and a variety of biologically important compounds)

rDNA technology – enabling us to produce improved varieties of plants and animals such as pest-resistant crops and may even enable us to correct genetic defects in human beings.

**Human genome project:**

• Microbial genetic techniques have made possible a colossal scientific undertaking HGP. Begun in 1990 and supposed to complete by 2005 but was completed in May 2000.

• Humans have just over 30,000 genes instead of estimates that ranged up to 142,000 genes. 3 billion base pairs in the human genome do not all code for useful genes (75% of them code for ‘junk DNA’)

• Over 100 microbial genomes have been sequenced so far.

• Approx. 113 genes have come to human genome directly from bacteria.

• Venter has sequenced mouse genome and reports that humans have only 300 genes not found in the mouse.

**MEDICAL MICROBIOLOGY**

Infectious diseases are caused by subcellular infectious entities (prions,viruses), prokaryotic bacteria, eukaryotic fungi and protozoans, metazoa animals, such as parasitic worms (helminths), and some arthropods. Definitive proof that one of these factors is the cause of a given infection is demon- strated by fulfillment of the three Henle-Koch postulates.

**Prions**

These are proteinaceous infectious particles. Evidence indicates that prions are protein molecules that cause degenerative central nervous system (CNS) diseases such as Creutzfeldt-Jakob disease, kuru, scrapie in sheep, and bovine spongiform encephalopathy (BSE) (general term: transmissible spongiform encephalopathies [TSE]).

**Viruses.**

Are Ultra-microscopic, obligate intracellular parasites that: — contain only one type of nucleic acid, either DNA or RNA.

They possess no enzymatic energy-producing system and no protein-synthesizing apparatus,

They force infected host cells to synthesize virus particles.

**Bacteria.**

These organisms reproduce asexually by binary transverse fission. They do not possess the nucleus typical of eucarya. The cell walls of these organisms are rigid (with some exceptions, e.g., the mycoplasma). & Chlamydiae. These organisms are obligate intracellular parasites that are able to reproduce in certain human cells only and are found in two stages: the infectious, nonreproductive particles called elementary bodies (0.3 lm) and the noninfectious, intracytoplasmic, reproductive forms known as initial (or reticulate) bodies (1 lm). & Rickettsiae. These organisms are obligate intracellular parasites, rodshaped to coccoid, that reproduce by binary transverse fission. The diameter of the individual cell is from 0.3–1 lm.

Nuclear structure and Circular DNA molecule not covered with proteins Complex of DNA and basic proteins; there is no localization of nuclear structure,instead there is a dense tangle of DNA in cytoplasm; no nuclear membrane; nucleoid or nuclear equivalent. No mitochondria and no endoplasmic reticulum. Cell wall Usually rigid wall with murein layer; exception: mycoplasmas Reproduction Asexual, by binary transverse fission In most cases sexual,

**Mycoplasmas.**

Mycoplasmas are bacteria without rigid cell walls. They are found in a wide variety of forms, the most common being the coccoid cell (0.3–0.8 lm). Threadlike forms also occur in various lengths.

**Fungi**.

Fungi (Mycophyta) are nonmotile eukaryotes with rigid cell walls and a classic cell nucleus. They contain no photosynthetic pigments and are heterotrophic, that is, they utilize various organic nutrient substrates (in contrast to carbon autotrophic plants). Of more than 50 000 fungal species, only about 300 are known to be human pathogens. Most fungal infections occur as a result of weakened host immune defenses.

**Protozoa**.

Protozoa are microorganisms in various sizes and forms that may be free-living or parasitic. They possess a nucleus containing chromosomes and organelles such as mitochondria (lacking in some cases), an endoplasmic reticulum, pseudopods, flagella, cilia, kinetoplasts, etc. Many parasitic protozoa are transmitted by arthropods, whereby multiplication and transformation into the infectious stage take place in the vector.

**Helminths**.

These are mainly the Parasitic worms that belong to the animal kingdom. These are metazoan organisms with highly differentiated structures. Medically significant groups include the trematodes (flukes or flatworms), cestodes (tapeworms), and nematodes (roundworms).

**MICROBIOLOGICAL FACTORS RELATED TO PATHOGENICITY**

The factors determining the genesis, clinical picture and outcome of an infection include complex relationships between the host and invading organisms and differ widely depending on the pathogen involved. Despite this variability, a number of general principles apply to the interactions between the invading pathogen with its aggression factors and the host with its defenses. Since the pathogenesis of bacterial infectious diseases has been researched very thoroughly, the following summary is based on the host–invader interactions seen in this type of infection.

There are five groups of potential bacterial contributors to the pathogenesis of infectious diseases:

1. Adhesins. They facilitate adhesion to specific target cells.
2. Invasins. They are responsible for active invasion of the cells of the macro- organism.
3. Impedins. These components disable host immune defenses in some cases.
4. Aggressins. These substances include toxins and tissue-damaging en- zymes.
5. Modulins. Substances that induce excess cytokine production (i.e., lipo- polysaccharides of Gram-negative bacteria, superantigens, murein fragments).

**Adhesion**

When pathogenic bacteria come into contact with intact human surface tis- sues (e.g., mucosa), they contrive to adhere to receptors on the surface of the target cells by means of various surface structures of their own (attachment pili, attachment fimbriae, adhesion proteins in the outer membrane of Gram- negative bacteria, cell wall-associated proteins in Gram-positive bacteria). This is a specific process, meaning that the adhesion structure (or ligand) and the receptor must fit together like a key in a keyhole.

#### Invasion and Spread

Invasion. Bacteria may invade a host passively through microtraumata or macrotraumata in the skin or mucosa. On the other hand, bacteria that invade through intact mucosa first adhere to this anatomical barrier, then actively breach it. Different bacterial species deploy a variety of mechanisms to reach this end:

Production of tissue-damaging exo-enzymes that destroy anatomical barriers.

Parasite-directed endocytosis, initiated by invasins on the surface of the bacterial cells, causes the cytoskeleton of the epithelial cell to form pseudopods that bring about endocytosis.

The Spread.

Local tissue spread beginning at the portal of entry, helped along by tissue-damaging exoenzymes (hyaluronidase, collagenase, elastase, and other proteases).

Cell-to-cell spread. Bacteria translocated into the intracellular space by endocytosis cause actin to condense into filaments, which then array at one end of the bacterium and push up against the inner side of the cell membrane. This is followed by fusion with the membrane of the neighboring tissue cell, whereupon the bacterium enters the new cell (typical of *Listeria* and *Shigella*).

Translocation of macrophage-resistant bacteria with macrophages into intestinal lymphoid tissue following their ingestion by M cells.

**Antiphagocytosis**

*Capsule*. Renders phagocytosis more difficult. Capsule components may block alternative activation of complement so that C3b is lacking (ligand for C3b receptor of phagocytes) on the surface of encapsulated bacteria. Microorganisms that use this strategy include *Streptococcus pneumoniae* and *Haemophilus influenza*

*Phagocyte toxins*. Examples: leukocidin from staphylococci, streptolysin from streptococci.

Serum resistance. Resistance of Gram-negative bacteria to complement. A lipopolysaccharide in the outer membrane is modified in such a way that it cannot initiate alternative activation of the complement system. As a result, the membrane attack complex (C5b6789), which would otherwise lyse holes in the outer membrane, is no longer produced

**Immunotolerance.**

* *Prenatal infection*. At this stage of development, the immune system is un- able to recognize bacterial immunogens as foreign.
* *Molecular mimicry*. Molecular mimicry refers to the presence of molecules on the surface of bacteria that are not recognized as foreign by the im- mune system. Examples of this strategy are the hyaluronic acid capsule of *Streptococcus pyogenes* or the neuraminic acid capsule of *Escherichia coli* K1 and serotype B *Neisseria meningitidis*.

**Antigen variation.** Some bacteria are characterized by a pronounced variability of their immunogens (= immune antigens) due to the genetic variability of the structural genes coding the antigen proteins. This results in production of a series of antigen variants in the course of an infection that no longer “match” with the antibodies to the “old” antigen. Examples: gonococci can modify the primary structure of the pilin of their attachment

**IgA proteases**. Mucosal secretions contain the secretory antibodies of the sIgA1 class responsible for the specific local immunity of the mucosa. Classic mucosal parasites such as gonococci, meningococci and *Haemophilus influenzae* produce proteases that destroy this immunoglobulin.

#### Clinical Disease

The clinical symptoms of a bacterial infection arise from the effects of dama- ging noxae produced by the bacteria as well as from excessive host immune responses, both nonspecific and specific. Immune reactions can thus poten- tially damage the host’s health as well as protect it (see Immunology, p. 103ff.).

Cytopathic effect. Obligate intracellular parasites (rickettsiae, chlamy- diae) may kill the invaded host cells when they reproduce.

Exotoxins. Pathogenic bacteria can produce a variety of toxins that are either the only pathogenic factor (e.g., in diphtheria, cholera, and tetanus) or at least a major factor in the unfolding of the disease. One aspect the classification and nomenclature of these toxins must reflect is the type of cell affected: cytotoxins produce toxic effects in many different host cells; neu- rotoxins affect the neurons; enterotoxins affect enterocytes.

* *Membrane toxins*. These toxins disrupt biological membranes, either by attaching to them and assembling to form pores, or in the form of phos- pholipases that destroy membrane structure enzymatically.
* *Superantigens:* These antigens stimulate T lymphocytes and macrophages to produce excessive amounts of harmful cytokines.

Hydrolytic exoenzymes. Proteases (e.g., collagenase, elastase, nonspecific proteases), hyaluronidase, neuraminidase (synonymous with sialidase), lec- ithinase and DNases contribute at varying levels to the pathogenesis of an infection.

**Secretion of virulence proteins.**

Proteins are synthesized at the ribosomes in the bacterial cytoplasm. They must then be secreted through the cytoplasmic membrane, and in Gram-negative bacteria through the outer membrane as well. The secretion process is implemented by complex protein secretion systems (I-IV) with differing compositions and functional path- ways. The type III (virulence-related) secretion system in certain Gram-neg- ative bacteria (*Salmonella*, *Shigella*, *Yersinia*, *Bordetella*, *Escherichia coli*, *Chla- mydia*) is particularly important in this connection

###### **Defenses against Infection**

A macroorganism manifests defensive reactions against invasion by microor- ganisms in two forms: as specific, acquired immunity and as nonspecific, innate resistance

#### Nonspecific Defense Mechanisms

Primary defenses. The main factors in the first line of defense against in- fection are mechanical, accompanied by some humoral and cellular factors. These defenses represent an attempt on the part of the host organism to pre- vent microorganisms from colonizing its skin and mucosa and thus stave off a generalized invasion.

Secondary defenses. The second line of defense consists of humoral and cellular factors in the blood and tissues, the most important of which are the professional phagocytes.

Phagocytosis. “Professional” phagocytosis is realized by polymorphonu- clear, neutrophilic, eosinophilic granulocytes—also known as microphages— and by mononuclear phagocytes (macrophages). The latter also play an im- portant role in antigen presentation (see p. 62). The total microphage cell count in an adult is approximately 2.5X 1012. Only 5 % of these cells are located in the blood. They are characterized by a half-life of only a few hours. Microphages contain both primary granules, which are lysosomes containing lysosomal enzymes and cationic peptides, and secondary granules. Both microphages and macrophages are capable of ameboid motility and chemotactic migration, i.e., directed movement along a concentration gradient toward a source of chemotactic substances, in most cases the complement compo- nents C3a and C5a. Other potentially chemotactic substances include secretory products of lymphocytes, products of infected and damaged cells or the *N*-formyl peptides

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**The Most Important Mechanisms in Nonspecific Defenses Against Infection**

1. **Mechanical factors**
2. Anatomical structure of skin and mucosa Mucus secretion and mucus flow from mucosa
3. Mucociliary movement of the ciliated epithelium in the lower respiratory tract Digestive tract peristalsis
4. Urine flow in the urogenital tract

**Humoral factors**

1. Microbicidal effect of the dermal acidic mantle, lactic acid from sweat glands, hydrochloric acid in the stomach, and the unsaturated fatty acids secreted by the sebaceous glands
2. Lysozyme in saliva and tear fluid: splitting of bacterial murein Complement (alternative activation pathway)
3. Serum proteins known as acute phase reactants, for example C-reactive protein, haptoglobin, serum amyloid A, fibrinogen, and transferrin (iron-binding protein)
4. Fibronectin (a nonspecific opsonin); antiviral interferon
5. Mannose-binding protein: binds to mannose on the outer bacterial surface, thus altering the configuration and triggering alternative activation of complement

**Cellular factors**

1. Normal flora of skin and mucosa
2. Natural killer cells (large, granulated lymphocytes; null cells)
3. Professional phagocytes: microphages (neutrophilic and eosinophilic granulo- cytes); mononuclear phagocytes (macrophages, monocytes, etc.) eosinophilic granulocytes); mononuclear phagocytes (macrophages, monocytes, etc.)

Phagocytes are capable of ingestion of both particulate matter (phagocytosis) and solute matter (pinocytosis). Receptors on the phagocyte membrane initiate contact (Fig. 1.6). Particles adhering to the membrane are engulfed, ingested and deposited in a membrane-bound vacuole, the so-called phago- some, which then fuses with lysosomes to form the phagolysosome. The bac- teria are killed by a combination of lysosomal factors

#### Specific Defense Mechanisms

Specific immunity, based on antibodies and specifically reactive T -lymphocytes, is acquired in a process of immune system stimulation by the corresponding microbial antigens. Humoral immunity is based on antitoxins, opsonins, microbicidal antibodies, neutralizing antibodies, etc. Cellular immunity is based on cytotoxic T lymphocytes (T- killer cells) and T- helper cells.

#### Defects in Immune Defenses

Hosts with defects in their specific and/or nonspecific immune defenses are prone to infection.

**Primary defects.** Congenital defects in the complement-dependent phagocytosis system are rare, as are B and T lymphocyte defects.

**Secondary defects.** Such effects are acquired, and they are much more frequent. Examples include malnutrition, very old and very young hosts,

1. Metabolic disturbances (diabetes, alcoholism),
2. Autoimmune diseases,
3. Malignancies (above all lymphomas and leukemias)
4. Immune system infections (HIV),
5. Severe primary diseases of parenchymatous organs injury of skin or mucosa,
6. Immunosuppressive therapy with corticosteroids,
7. Cytostatics and immuno- suppressants, and radiotherapy.

One result of progress in modern medicine is that increasing numbers of patients with secondary immune defects are now receiving hospital treatment. Such “problem patients” are frequently infected by opportunistic bacteria that would not present a serious threat to normal immune defenses. Often, the pathogens involved (“problem bacteria”) have developed a resistance to numerous antibiotics, resulting in difficult courses of antibiotic treatment in this patient category.

###### **Transmission, Sources of Infection**

Transmission

Microbiological pathogens can be transmitted from a source of infection by direct contact or indirectly.

Person-to-person transmission constitutes a homologous chain of infection. The infections involved are called anthroponoses. In cases in which the pathogen is transmitted to humans from other vertebrates (and occasionally the other way around) we have a heterologous chain of infection and theinfections are known as zoonoses

|  |  |
| --- | --- |
| **Direct transmission** | **Indirect transmission** |
| Fecal-oral (smear infection) |  |
| Aerogenic transmission (droplet infection). | Transmission via food |
| Genital transmission (during sexual intercourse). | Transmission via drinking water. |
| Transmission via skin (rare) | Transmission via contaminated inanimate objects or liquids. |
| Diaplacental transmission | Transmission via vectors (arthropods). |
| Perinatal transmission (in the course of birth | Transmission via other persons (e.g., via the hands of hospital medical staff). |

**Confronting Micro-Organisms and infection**

Confronting and preventing infectious diseases can sometimes involve substantial incursions into the private sphere of those involved as well as economic consequences. The centerpiece of every disease prevention system is provision for re- porting outbreaks. Basically, reporting is initiated at the periphery (individual patients) and moves toward the center of the system. Concrete countermeasures in the face of an epidemic take the form of prophylactic measures aimed at interrupting the chain of infection.

#### Exposure Prophylaxis

Exposure prophylaxis begins with *isolation* of the source of infection, in particular of infected persons, as required for the disease at hand. *Quarantine* refers to a special form of isolation of healthy first-degree contact persons. These are persons who have been in contact with a source of infection. The quarantine period is equivalent to the incubation period of the infectious disease in question Further measures of exposure prophylaxis include *disinfection* and *steri- lization*, use of insecticides and pesticides, and eradication of animal carriers.