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Vitebsk State Medical University
Department of Clinical Microbiology

I.I. Generalov

MEDICAL MICROBIOLOGY IN DENTISTRY

LECTURE COURSE

Textbook for students of medical universities, Faculty of Dentistry

The author: Generalov I.I.

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Reviewed by:
I.V.Zhyltsov, MD, Dr.Sci, Professor of Dpt of Infectious Diseases,
Vitebsk State Medical University

O.D. Myadeletz, MD, Dr.Sci, Professor, Head of Dpt of Histology,
Vitebsk State Medical University

Generalov I.I.

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Lecture course on medical microbiology in dentistry comprises the main topics covering the most common dental diseases, associated with infection, the principles of their pathogenesis, laboratory diagnosis, prophylaxis and treatment. The book is prepared according to basic educational plan and program, approved by Ministry of Education and Ministry of Health Care of Republic of Belarus. The edition is assigned for students studying at Faculties of Dentistry of medical universities.

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CONTENTS

ABBREVIATION LIST

Ab, Abs – antibody, antibodies
Ag, Ags – antigen, antigens
AIDS – acquired immunodeficiency syndrome
ANUG – acute necrotizing ulcerative gingivitis
APC – antigen-presenting cells
CADIS – candida-associated denture induced stomatitis
CD – cluster of differentiation (or cluster of designation)
Da – dalton; atomic (molecular) mass unit
DC – dendritic cells
DIC – disseminated intravascular coagulation
DPT – diphtheria-pertussis-tetanus vaccine
ELISA – enzyme-linked immunosorbent assay
FAE cells – follicule-associated epithelial cells
GALT – gut-associated lymphoid tissue
GPAC – gram-positive anaerobic cocci
HAART – highly active antiretroviral therapy
HIV – human immunodeficiency virus
HLA – human leukocyte antigens
HSV – herpes simplex virus
IgA (G, M) – immunoglobulin A, (G, M)
IL – interleukin
LPS – lipopolysaccharide (bacterial)
MAC – membrane-attack complex (of complement)
M cells – microfold cells
MDR – multi-drug resistant tuberculosis
MG – mucin glycoprotein
MHC – major histocompatibility complex
MODS – multiple-organ-dysfunction syndrome
MRSA – methicillin resistant *Staphylococcus aureus*
NAATs – nucleic acid amplification tests
NALT – nasal mucosa-associated lymphoid tissues
NK cells – natural killer cells
PCR – polymerase chain reaction
ROI – reactive oxygen intermediates
SIRS – systemic inflammatory response syndrome
Tc – T cytotoxic cells
Th – T helper cells
VAP – ventilator-associated pneumonia
XDR – extensively drug-resistant tuberculosis

I. NORMAL MICROFLORA OF ORAL CAVITY

1.1. General characteristics of oral microbiota

Oral cavity provides favorable conditions for growth and propagation of multiple microbial inhabitants. They can be found in great amounts on mucous membranes of tongue, cheeks, teeth, gingival crevices and pockets. Species composition of oral microflora is extremely variable (see Table 1).

Table 1.
Typical representatives of oral microbiota

Microbial species	In saliva		In gingival crevices (detection rates and grades)
	Detection rates, %	*Quantity, cells/ml*	
Group A. Resident autochthonous microflora			
I. Aerobic and facultatively anaerobic:			
S. mutans	100	$1,5*10^5$	100
S. salivarius	100	10^7	100
S. mitis	100	10^6-10^8	100
Saprophytic neisseriae	100	10^5-10^7	++
Lactobacilli	90	10^3-10^4	+
Staphylococci	80	10^3-10^4	++
Diphtheria-like corynebacteria	80	No data	+
Actinomycetes	100	No data	++
Candida and other yeast-like fungi	50	10^2-10^3	+
Mycoplasmas		10^2-10^3	No data
II. Obligate anaerobes			
Veilonellas	100	10^6-10^8	100
Anaerobic streptococci (peptostreptococci)	100	No data	100
Бактероиды	100	No data	100
Fusobacteria	75	10^3-10^4	100
Group B. Transient allochthonous microflora			
Aerobic and facultatively anaerobic:			
Gram-negative rods			
Klebsiella spp.	15	10-10^2	0
Aerobacter spp.	3	10-10^2	0

Oral cavity harbors even more than 500-600 of diverse bacterial species. Their absolute quantity is enormously high as well. For instance, total salivary microbial count exceeds 1 billion cells per 1 ml.

These bacteria encompass mixed microflora from various compartments of oral cavity. Most of them participate in dental plaque formation.

There are two main groups of bacteria that make oral microbiota – *autocthonous* and *allochthonous* microflora.

Autochthonous or ***indigenous*** bacteria are the resident inhabitants of oral cavity (*obligate* microflora), whereas *allochthonous* microorganisms are temporary for this site (or *transient*) arising largely from external source. Nevertheless, transient oral microflora comprises more likely pathogenic and opportunistic bacterial species in comparison with resident ones.

Allochthonous microorganisms enter oral cavity from other biotopes of human body (e.g., large intestine) or from external environment.

The group of resident aerobic and facultatively anaerobic gram-positive cocci encompasses mainly ***viridans streptococci***. They produce green zone of hemolysis when grown onto blood agar medium. Most common here are *S. mutans, S. mitis, S. sanguis, S. salivarius*. Their quantitative distribution depends on many variable external and internal factors: person's diet, oral cavity personal hygiene, state of local immune response, genetic factors, etc.

Streptococci can produce hydrogen peroxide and ferment carbohydrates yielding organic acids. This lowers local pH below 5.0 resulting in dental enamel demineralization and teeth decay. Furthermore, streptococci are capable of making polysaccharides from sucrose taken from sucrose-containing foodstuffs. Soluble oligosaccharides are metabolized by other bacteria thereby intensifying acid formation. Non-soluble polysaccharides actively promote adhesion of oral streptococci to dental surface thus fostering dental plaque growth.

Gram-positive anaerobic cocci are represented by peptococci that intensively utilize peptides and amino acids. Unlike streptococci they demonstrate slow carbohydrate fermentation.

Resident oral gram-negative anaerobic cocci, e.g., *Veillonella* genus members, play important role in metabolic balance within oral cavity. They don't ferment mono- and disaccharides but utilize numerous organic acid (lactate, pyruvate, acetate and others) yielding CO_2 and H_2O end products. This leads to acid content neutralization and pH rise that ameliorates local environment. Taking into account virtually similar amount of viridans

streptococci and veillonellas in saliva the latter degrade lactic acid produced after streptococcal fermentation thus protecting against caries.

Gram-negative diplococci from *Neisseria* genus are facultatively anaerobic. They can be found at early stage of dental plaque initiation and growth. Unlike streptococci they demonstrate slow rate of propagation. Their most common species are *N. sicca* that produce various polysaccharides and *N. subflava.*

Oral gram-positive aerobic and facultatively anaerobic rods comprise lactobacilli, corynebacteria and some other representatives.

The members of *Lactobacillus* genus generate ample quantities of lactic acid upon carbohydrate fermentation that actively stimulates caries progression. Corynebacteria lower redox potential in local dental surroundings ensuring beneficial conditions for anaerobic bacteria overgrowth (e.g., bacteroids, prevotellas, porphyromonads, fusobacteria, spirochetes, and many others). Moreover, corynebacteria produce vitamin K that is used as potent growth factor by many oral bacteria.

Two genera from *Actinomycetaceae* family, namely *Actinomyces* and *Bifidobacterium*, can be found in oral microflora as well.

Actinomycetes easily settle upon mucous layer of oral cavity; they are typical microbial constituents of dental plaque and dental stone. Actinomycetes are commonly isolated from ducts of salivary glands, gingival pockets, and carious cavities. These bacteria possess weak proteolytic activity but intensively ferment carbohydrates accumulating broad spectra of organic acids (lactate, acetate, succinate, formate and others).

The species *A. israelii*, *A. naeslundii genospecies 2* (former A. *viscosus*) contribute to caries and periodontal disease progression.

Bifidobacteria ferment numerous carbohydrates with lactic and acetic acid end products predisposing to decay of dental enamel and caries.

Gram-negative rods predominantly comprise obligate anaerobic bacteria from genera *Bacteroides, Porhyromonas, Prevotella, Fusobacteium, Leptotrichia.* These agents are autochthonous representatives of oral microbiota. They lack of catalase, ferment carbohydrates with gas and hydrolyze proteins to amino acids.

Multiple *bacteroidal* members of microbial community belong to *B. forsythus*, *B. gracilis*, *B. urealyticus*, and many other species. In association with streptococci and fusobacteria they may exert periodontal disorders.

Pigment bacteria *Porphyromonas gingivalis* and *P. endodontalis* are isolated from periodontal tissues. They are typically indole-producing.

P. gingivalis expresses collagenase that acts detrimentally on dentin layer and destroys fibrinogen. These pathogens are found in gingivitis and periodontal pathologies.

Very common oral pathogens are *Prevotella melaninogenica, P. oralis, P. denticola, P. buccalis*. Their carbohydrate-fermenting capacity is low. *P. melaninogenica* is the constant habitant of dental pockets in adults. By secretion of phospholipase A it breaks cell membrane integrity thereby stimulating periodontal diseases.

Fusobacteria are the spindle-like polymorphic rods that grow in dental pockets in association with other bacteria (e.g., spirochetes). They weakly ferment carbohydrates and peptone, releasing butyric, and lesser amounts of lactic and acetic acids; prodice indole. Typical representative is *F. nucleatum.*

Leptotrychia are granular polymorphic rods, some of them are filamentous. Most frequent agent here is *L. buccalis*, capable of glucose fermenting with large amounts of lactic acid. The amount of these bacteria raises in case of periodontal disease progression..

Conventional members of dental microflora embrace numerous spirochetal species of *Treponema*, *Borrelia* and *Leptospira* genera. Typical oral treponemas are *T. oralis, T. macrodentium, T. denticola* and others.

Treponema vincentii is ordinarily found in oral folds and dental pockets. It produces modest amounts of acetic and butyric acids. In persons with weakened immunity *Treponema vincentii* together with prevotellas and fusobacteria exerts acute necrotizing ulcerative gingivitis (or ANUG), demonstrating sudden onset.

Gingival pockets often harbor *Borrelia buccalis* – large spirochetes that live in symbiotic associations with fusiform bacteria.

Most of oral mycoplasmas pertain to saprophytic *M. orale* and *M. salivarium* species.

Candida fungi participate in colonization of oral mucosa in closest interrelationships with neighboring bacteria. In most situations they don't evoke pathological changes. However, in cases of indiscriminate use of antibiotics or secondary immune deficiencies *oral candidiasis* can arise thus indicating deep shift in local microbiota composition that results in dysbiosis.

1.2. Ontogenesis of normal oral microflora

Bacterial entry to newborn's oral cavity occurs initially at the time of delivery. Primary oral microflora is composed of lactobacilli, enterococci,

micrococci, staphylococci and some others. In first weeks these casual microorganisms will be displaced by certain bacterial species inhabiting maternal oral cavity. Likewise, medical personnel of obstetrics care settings becomes the next source of microbial contamination. Aerobic and facultatively anaerobic microflora dominates in newborn's oral cavity. Among them are streptococci, lactobacilli, neisseriae and candida fungi. Their total count rises up to 4th month of life; then it gradually declines. Initial amount of anaerobic bacteria is very low (veillonellas, fusobacteria, and some others). Usually they stay within the folds of oral mucous membranes.

Dentition creates new opportunities for anaerobic bacteria propagation. Anaerobes begin to spread throughout the all compartments of oral cavity. At puberty the number of anaerobic bacteria arises; bacteroids, prevotellas and spirochetes become typical this time.

In elderly people with multiple comorbidity and lowered immunity the composition of normal oral microflora is profoundly altered. The number of staphylococci as well as candida fungi elevates substantially; *E. coli* and enterococci can be found. The presence of removable dental prosthetic devices facilitates the shift in microbial composition resulting in emergence of prosthetic stomatitis. The plaques made of settled microorganisms and organic matrix under partial dentures accumulate acidic substances that favor candidal propagation. Oral candidiasis in patients with dental prostheses can occur in more than 70% of cases. In these situations candidae may spread from initial colonization site towards any oral compartment. In fact, they cause angular stomatitis when located in angulus oris.

Similarly, the bacteria colonizing oral cavity, can afflict airways and gastrointestinal tract.

1.3. Microflora of saliva, tongue, dental plaque, and gingival crevices

Saliva and gingival crevicular fluid are the main liquid substances washing oral cavity. The saliva is crucial in balancing oral microbial ecology. All the properties of saliva (secretion rate, viscosity, mineral contents, ionic potential, buffering capacity, multiple organic matter – amino acids, polysaccharides, vitamins, nucleotides, potent antimicrobial factors – mucins, secretory IgA antibodies, lyzozyme) contribute to microbial composition of oral cavity.

Besides saliva, the bacteria are located preferentially in three zones of oral cavity: dental plaques upon tooth crown (or inside carious lesions in case of caries); within gingival crevices; and upon lingual body especially covering its back side.

Total amount of bacteria in saliva varies in the range from 40-50 mln to more than 5 billion per 1 ml, for about 750 mln an average. Microbial concentration in dental plaques and gingival crevices is almost 100-fold higher – nearly 200 bln microbial cells per 1 g of medium content. Besides microbial cells, the latter harbors about 80% of water.

As mentioned above numerous microbial species and genera reside in oral cavity. More than one-half pertain to vast number of streptococcal species, e.g., *S. mutans*, *S. mitis*, *S. sanguis*, *S. sobrinus* and others – except beta-hemolytic streptococci that can be found solely as transient microflora. Various coccal species occupy certain compartments within the mouth. For instance, most of enterococci are located inside gingival crevice and upon the body of tongue; *S. mutans* is typically found in dental plaque upon crown.

Viridans streptococci and veillonellas produce the great mass of salivary microflora. Mostly they shed there from tongue body. The number of gram-negative anaerobic rods (bacteroidal species and fusobacteria) together with diphtheroids increases in gingival crevices.

Total quantity of microbial cells undergoes daily alterations. It depends mainly on amount of saliva secretion that greatly declines at night. Dental loss leads to marked reduction of dental microflora.

Multiple factors can impact the certain members of oral microbiota. For example, any antibiotic treatment inhibits the target group of defined microbial species thus impairing normal microbial balance. Protein-enriched diet doubles the number of facultatively anaerobic gram-positive rods. Large part of bacteria needs vitamins or other supplements for their successful propagation; lack of growth factors results in suppression of activity of selected bacterial groups.

Qualitative and quantitative composition of dental microflora is greatly influenced by various diseases. For instance, *C. albicans* recovery from oral samples is made with highest rate in diabetes patient (up to 80% against 50% in healthy individuals). Lactobacilli grow high in caries patients and fall down after lesions treatment.

It can be indicated also that *S. mutans*, *S. sanguis*, lactobacilli, yeasts and spirochetes seriously disappear after massive dental loss, whereas the amount of *S. salivarius* elevates in the course of time. In first 2 weeks after mounting of removable dentures the levels of streptococci look high,

whereas the quantity of lactobacilli and yeasts rapidly goes down. In 3-5 weeks the count of lactobacilli and yeasts tends to restore but the number of streptococci declines. Overall, the total number of streptococci doesn't alter significantly in all periods of life.

Polymicrobial adherence to dental surface leads to *dental plaque* formation.

Dental plaque is a complex matrix (or ***microbial biofilm***) made of immensity of microbial bodies, their extracellular products and wastes, and salivary compounds.

Dental plaques are divided into ***supragingival*** and ***subgingival.*** Supragingival plaques play substantial role in caries. Likewise, subgingival palques participate in periodontal disease progression.

Composition of dental plaque differs depending on site of adherence and plaque's maturation stage. It grows predominantly on dental surfaces that avoid mechanical cleaning – approximal surface between two teeth, fissures and pits of the tooth crowns, gingival crevices

The process of plaque formation commences from adhesion of poorly soluble polymeric carbohydrates such as dextrans together with mucins and salivary proteins to dental enamel. Acid glycoproteins react with calcium ions of enamel whereas basic proteins bind to phosphates of hydroxyapatites. Primary biofilm is known as ***pellicle.***

Attachment of bacteria demonstrates rapid progression. By 5 minutes the number of microbes arises up to 10^5-10^6 of bacterial cells per 1 cm^2. Initial microbial bodies land within tooth pits and fissures; later they spread to smooth dental areas. Further microbial propagation and their exopolysaccharide excretion facilitate the growth of soft dental plaque.

Many bacterial cells can't attach firmly to clean dental surface but easily bind to primarily absorbed microbial layer. For instance, when coccoidal flora surrounds embedded rod-like and filamentous bacteria, it produces mixed cellular clusters known as *corncob formations*.

The bacteria composing dental plaque can be divided into two large groups. The first comprises acidophilic agents able to propagate in acidic environment – lactobacilli, actinomycetes, peptococci, leptotrichia, corynebacteria and some others. The second one embraces bacteria with prominent proteolytic activity – veillonellas, fusiform bacteria, neisserias, vibrios, or spirochetes.

At initial steps of maturation the dental plaque has larger amounts of aerobic and facultatively anaerobic bacteria with dominating role of oral streptococci.

Oral viridance streptococci together with lactobacilli ferment sucrose resulting in overproduction of lactic acid and next sharp decline of local oral pH. Lactate can be further utilized by veillonellas, neisserias and other microbials accumulating more organic acids (eg. acetic, propionic or formic). All these changes influence microbial composition of dental plaque.

Exuberant consumption of sucrose and other simple carbohydrates from nutrients worsens the situation and intensifies enamel destruction, microbial retention and plaque maturation. In addition, elevated levels of carbohydrates in oral cavity lead to their polymerization by local microbiota. Synthesis of extracellular polysaccharides such as soluble or insoluble dextran and levan is typical for oral streptococci especially *S. mutans*. They facilitate microbial tooth adhesion and consolidate the matrix of microbial biofilm within dental plaque.

The synthesis of bacterial exopolysaccharides ceases at pH below 5,0.

Supragingival dental plaque predominantly harbors facultatively anaerobic gram-positive bacteria, mainly the broad spectra of streptococci and actinomycetes. Gram-negative representatives that pertain to *Veillonella*, *Bacteroides* and *Haemophilus* species are present constantly but in lower concentrations.

Similarly, subgingival dental plaque also confines the most common gram-positive microorganisms – streptococci and *Actinomyces spp.* Non-affected subgingival crevice carries moderate number of microbes; their total number varies from 10^3 to 10^6 cells per site.

Composition of bacterial plaques is different also on teeth of upper and lower jaws. A large proportion of streptococci and lactobacilli is present within dental plaques of upper jaw. Veillonellas and filamentous bacteria can be often found on teeth of mandibular bone.

Gram-positive cocci and rods prevail on approximal dental surfaces (between teeth) and within fissures. First day of plaque emergence is characterized by swift primary microbial colonization. After plaque maturation their microbial composition remains stable for a long time.

Next plaque progression is followed by lowering of its redox potential under the action of aerobic and facultatively anaerobic bacteria, thus engendering the growth of obligate anaerobic organisms. The dental plaque progressively accumulates bacteroids, porhyromonads, prevotellas, fusobacteria, leptotrichias and many others. Their metabolism results in alkaline byproducts (e.g., ammonia, urea, etc.) thereby elevating dental pH and dampening further plaque growth.

Sequential change of microbial communities, basic character of elderly plaque biofilm, accumulations of calcium and phosphates predispose to the formation of *dental stone* (*calculus*, or *tartar*). It begins to grow on dental surface especially in the area of gingival margin that impedes circulation of crevicular fluid.

Dental stone (***calculus***) is the solid formation tightly attached to dental crown and/or radix that is resulted from consolidation and calcification of contents of long-term dental plaque (degraded microbial bodies and polymeric matrix, inorganic matter, etc.)

Dental stones are also divided into ***supragingival*** and ***subgingival.*** *Supragingival stones* can be ordinarily found nearby the openings of ducts of salivary glands or upon the lingual surface of lower molars. *Subgingival* attach to dental radices. This stimulates progression of dental pockets impacting gum detachment.

Overall, dental plaques and dental stones impair the normal self-cleaning of dental areas and promote the development of most common aggressive disorders – i.e., *caries* and *periodontitis*.

Efficient prophylaxis of these widespread dental diseases depends on the number of medical and hygienic measures for prevention and removal of dental plaques such as brash cleaning of teeth and dental flossing; the use of proper tooth pastes and powders that ensure plaque withdrawal.

1.4. Biological role of normal oral microbiota

Indigenous microflora of healthy oral cavity predominantly comprises commensal non-pathogenic microorganisms. It performs a lot of essential activities supporting normal body physiology. For instance, it creates biological barrier blocking invasion and propagation of pathogenic microorganisms and stimulates lymphoid tissue maturation thereby taking part in host defense against infectious agents. Likewise, the members of normal oral microbiota render antagonistic activity against multiple pathogenic species, which have the portal of entry in oral cavity. Autochthonous microflora participates in self-clearance of oral cavity. Similarly to many other representatives of human gastrointestinal tract, the resident oral bacteria supply the body with vitamins and amino acids produced by bacterial cells. Other metabolic end products of normal microflora can stimulate secretion of salivary glands and mucous membranes fostering permanent wash of oral cavity. This facilitates food intake, oral food digestion, chewing and swallowing.

1.5. Dysbiosis of oral cavity

Oral microbiota looks like explicitly complex, multi-component and multi-functioning assemble of microbial communities that is characterized by contemporaneous cross-linked interplays between aerobic, facultatively anaerobic and obligate anaerobic bacteria with their multiple species of gram-positive and gram-negative agents. Stable but fragile equilibrium established within oral microbiota through the years of long evolution maintains the healthy state of oral cavity.

The shift in this balance may deeply deregulate normal metabolism and function of oral ecosystem.

For instance, *indiscriminate use of antimicrobial agents* (antibiotics or antiseptics) especially with broad-spectrum of activity can easily provoke oral ***dysbiosis*** (or ***dysbacteriosis***). It promotes the damage of oral mucosa resulting in drug-related stomatitis, glossitis or other ailments. These disorders are caused predominantly by *Candida* fungi, or sometimes by enterococci and various gram-negative rods.

The most common etiological agent of oral dysbiosis is yeast-like dimorphic fungal species *Candida albicans*. These fungi demonstrate enhanced adhesive capacity to epithelial cells. Carious excavations create efficient ecological niche for candidal colonization and successful propagation. Under long-term therapy with antibiotics of broad spectrum or following secondary immunodeficiencies candidal overgrowth results in ***candidiasis***. Fungal hydrolytic enzymes (e.g., numerous proteases, neuraminidase and others) take active part in its pathogenesis.

The interaction between yeasts and oral mucosal membranes starts from fungal adhesion. Sucrose, maltose, glucose and other sugars contribute to tight microbial adherence.

Adhesive capacity of fungal cells alternates depending on microbial strain. It is responsible for virulence of certain candidal isolate. The most pathogenic *C. albicans* absorb upon human epithelium approximately 1.5 times faster than the members of other species. Antibacterial treatment promotes yeast adherence whereas complement activation through fungal mannans seriously dampens it.

When propagating, yeast-like fungi take part in dental enamel decay and caries progression. Similarly, they may cause mycotic stomatites and tonsillitis.

In most pathological cases the fungi of *Candida* genus are associated with other microorganisms of oral cavity (*mixed infection*). Their synergistic interplays ensue from fungal synthesis of numerous growth

factors (namely, vitamins) that stimulate propagation of many bacterial species, e.g., lactobacilli or actinomycetes. By contrast, lactobacilli generate large amounts of lactic acid; and acidification of local environment suppresses candidal growth.

Thus, ordinary fungal colonization doesn't expand into visible pathology. Nevertheless, in conditions mentioned above (extensive antibiotic treatment or local immunodeficiency) latent fungal infection transforms into clinical *candidiasis* (typical ***opportunistic infection***). Predominant clinical forms of candidiasis are local oral lesions, but in severe cases they grow into devastating generalized mycosis with multiple organ failure.

II. NON-IMMUNE AND IMMUNE DEFENSIVE MECHANISMS IN ORAL CAVITY

Normal or pathogenic microflora of oral cavity is constantly affected by wide network of defensive factors operating in this area. And vice versa, every microbial agent harbors countless antigenic ensemble that stresses all the sides of host defensive forces.

Once penetrated, a complex antigenic substance such as bacterial cell immediately triggers the vast set of *non-immune* and *immune reactions*. Notably, *immune reactions* can be both *non-specific* and *specific*. In most cases (in 90% and even more events) non-specific response is able to eliminate the limited number of pathogen invaded. But in other situations (massive microbial load, rapid pathogen penetration or toxin production) powerful specific immune reactions are activated.

2.1. Non-specific defensive factors of oral cavity

Decisive role in protection of oral tissues from harmful microbial activities is played by ***saliva*** and epithelial cells of ***mucous membranes*** with their ***barrier function***. Continuous salivary flow washes out carbohydrates from dental surfaces. Salivary glands produce 0.5 to 3 l of saliva per day. Saliva efficiently controls supragingival environment and the same way prevents microbial entry into subgingival space. The most powerful salivary bicarbonate buffer maintains pH of oral cavity within the range of 6.7-7.3. However, the diffusion of salivary components through dental plaque is slow; that's why pH in central part of dental plaque falls down to 5.0 or even less.

Oxidation-reduction or ***redox potential*** substantially influences growth and reproduction of microbial populations and the rates of enzyme reactions in oral cavity. The levels of redox potential depend strongly on local concentrations of molecular oxygen. Positive values of redox potential indicate aerobic conditions, negative – anaerobic ones. Saliva, tongue body, mucous epithelium of cheeks and palate have positive redox potential (+158-540 mB), dental crevices and approximal dental surfaces display negative potential – about (–300 mB).

The process of dental plaque maturation reduces local redox potential from (+294 мВ) to (–140 мВ). This leads to successful propagation of anaerobic bacteria.

Powerful ***innate immunity*** of oral cavity is maintained by multiple humoral and cellular non-specific immune reactions.

The ***system of humoral non-specific defense factors*** encompasses *mucins*, *glycoproteins*, *lactoferrin*, *lyzozyme*, *peroxidase*, short-chain basic defensive proteins *histatins* and *cystatins*. They are present in saliva and crevicular fluid in relatively large amounts.

Mucins are highly polymeric viscous glycoproteins secreted predominantly by submandibular and sublingual salivary glands. There are 2 major mucin glycoproteins in saliva – *MG1* and *MG2*.

MG1 has molecular weight more than 1 mln Da. It tightly covers mucous membranes of oral cavity. ***MG2*** of molecular weight for about 125 kDa hinders aggregation and adhesion of oral streptococci. Viscous mucin layer captures dental microflora thereby preventing bacterial penetration. Mucins also reduce acidic demineralization of hard dental tissues.

Likewise, other *salivary glycoproteins* block microbial adhesion to underlying oral mucous membranes.

Small cationic enzyme ***lyzozyme*** (or ***muramidase***) hydrolyses glycosidic bonds between N-acetyl-glucosamine and N-acetyl-muramic acid within bacterial cell wall *peptidoglycan* (or murein). It exerts easy lysis of bacterial cells.

Furthermore, lyzozyme binds to monovalent anions, e.g., perchlorates, iodides, bromides, fluorides, thiocyanates, and some others, and at the same time it binds to salivary proteases. This complex destabilizes the envelope of bacterial cells thus activating bacterial autolysins with subsequent cell lysis.

Lactoferrin is the iron-binding salivary glycoprotein that is synthesized by intercalated duct cells and granulocytes. Anti-microbial action of lactoferrin depends on its *specific iron-binding capacity*. Under binding it makes ferric ions unavailable for bacterial cells. Iron-deprived bacteria stop their growth and reproduction.

Iron-free lactoferrin or *apolactoferrin* demonstrates direct antibacterial effect agglutinating microorganisms that stimulate caries progression (*S. mutans*, *P. gingivalis*, *Aggregatibacter actinomycetemcomitans*).

Salivary peroxidase is secreted by acinar cells. It is thermostable enzyme active in broad pH range (3.0 to 7.0) and resistant to proteolysis. It inactivates hydrogen peroxide generated by oral microflora and diminishes

acid accumulation within dental plaque. Peroxidase retains stability being absorbed on hard dental tissues, e.g., enamel. As the result, this enzyme slows down the progression of dental plaque, caries and periodontal diseases.

Histatins are low molecular weight (3 to 5 kDa) antimicrobial salivary peptides. They comprise the group of small basic peptides secreted by acinar cells which are enriched with histidine.

Histatins block the growth of common oral pathogens (*S. mutans*, *C. albicans*), aggregation of porphyromonads and streptococci.

Cystatins pertain to one more family of salivary antimicrobial peptides. They diffuse to saliva from gingival crevicular fluid. *Cystatins* work as inhibitors of bacterial thiol proteases thereby impairing normal microbial metabolism.

Substantial part of salivary antimicrobial defense is related with ***complement system***. Complement proteins leak from gingival capillaries into crevicular epithelial cells and reach gingival crevice. Then in smaller amounts they may spread to saliva. This gradual overflow strongly accelerates in case of oral inflammation.

Complement *proteins* render the variety of defensive reactions against invaded pathogens: *lysis* of target cells (for instance, bacterial or viral-infected); *production of chemoattractants and mediators* that participate in inflammation and allergy, *opsonization* of bacteria and immune complexes for clearance by phagocytosis.

Complement activation can take one of three pathways. The first is the *classical pathway*, which is initiated by specific *antigen-antibody complexes*. The order of activation of *lectin pathway* closely resembles classical one except the primary step: lectin pathway is stimulated by reaction of host carbohydrate-binding proteins or *lectins* (e.g., human mannose-binding lectin) with bacterial polysaccharides.

The third route of complement activation is the *alternative pathway* that can be triggered by extremely diverse stimuli (e.g., components of bacterial cells, their endotoxins, host IgA molecules, some chemical substances, etc.) Complement activation via this pathway is the most common in oral cavity.

In all three pathways of complement activation the resulting membrane-attack complex or MAC exerts the lysis of microbial cells. Nevertheless, general conditions for complement action in oral cavity are not so beneficial as in bloodstream.

2.2. Biological activity of secretory IgA

Despite the evident protective power of all above-mentioned defensive mechanisms, it remains uncontested that ***secretory immunoglobulin A*** is clearly the most significant factor of humoral response in oral cavity. Secretory IgAs display their protective features by action on multiple targets: both specific and non-specific, humoral or cellular.

It must be pointed out that in large amounts IgA is present not only in saliva, but in milk, tears, colostrum, etc., thus being the dominant human immunoglobulin in secretions of the respiratory, intestinal, and genital tracts. It rids mucous membranes of attacks made by bacteria and viruses.

IgAs together with other immunoglobulin classes are produced by plasma cells. Plasma cells of secretory glands are located in their stromal part being adjusted to acini and ducts.

Every ***secretory IgA molecule*** (with molecular weight for about 350 kDa) consists of two *heavy* (H) and *light* (L) protein chains and one molecule each of ***J chain*** (15.6 kDa) and ***secretory component*** (70 kDa). J chain and secretory component have excessive amounts of carbohydrates in their structure.

Secretory component binds to IgA dimers and supports transportation of IgA molecules across mucosal epithelial cells towards the lumen of salivary ducts.

Some IgAs exist in serum as a monomeric $(H\text{-}L)_2$ molecule (MW 170,000). Serum concentration of IgA is about 1.0-4.0 g/l.

There are at least two IgA subclasses, namely IgA1 and IgA2. Salivary IgA1 subclass antibodies bind preferably to bacterial proteins and carbohydrates, whereas IgA2-subclass molecules react with lipoteichoic acids of gram-positive bacteria and lipopolysaccharide (or LPS) of gram-negative ones.

Some oral bacteria (e.g., streptococci and neisseriae) can destroy IgA1 by producing a specific protease thereby overcoming antibody-mediated resistance of mucosal surfaces.

Internal and external secretions of oral cavity are different in IgA content. Internal secretions are present in gingival crevices and pockets (e.g., crevicular fluid); and IgA/IgG ratio there is similar to serum proportions (nearby 1 to 6). On the contrary, IgA dominates within external secretions (saliva), where IgA/IgG ratio exceeds 100/1.

The induction of secretory IgA synthesis by plasma cells can be triggered locally in oral cavity or after the migration of stimulated B cells

from gut-associated lymphoid tissue (GALT) towards salivary glands and lymphoid tissue of oral cavity.

Secretory IgAs are endowed with multiple protective functions. They slow down microbial adherence, block the action of bacterial toxins, and neutralize viruses. They successfully perform so-called *immune exclusion*, preventing antigenic transposition from oral cavity through the epithelium of mucous membranes to regional lymph nodes and blood flow. For instance, sIgA antibodies inhibit the attachment of *S. mutans* to dental enamel thus breaking down the starting point for caries. Furthermore, sIgAs inactivate streptococcal glycosyltransferase enzyme that catalyzes exo-polysaccharide synthesis required for efficient bacterial adhesion. Similar action they demonstrate against fungal pathogen *C. albicans*.

Secretory IgAs foster antimicrobial activities of oral mucins, lactoferrin and salivary peroxidase mainly owing to extensive non-immune interactions of these substances with oral IgA molecules. In fact, it exerts tight cross-agglutination of adherent microbial cells thus limiting bacterial spread and propagation.

Finally, IgAs intensively stimulate several lines of immune cells via binding to Fc-receptors for IgA on membranes of macrophages, dendritic cells, granulocytes and T cells thereby triggering *antibody-dependent cell cytotoxicity* (or *ADCC*).

Sufficient levels of secretory IgA antibodies may prevent the flare-up of certain oral infections of viral etiology, e.g., herpetic exacerbations. In patients with selective IgA deficiency the viruses can easily reproduce in oral mucosa resulting in specific viral lesions.

2.3. Cellular innate immune responses in protection of oral cavity

Primary cellular reactions of *innate anti-bacterial immunity* are generally based on ***phagocytosis*** and activity of phagocytic cells within oral cavity. The latter comprise the members of ***mononuclear phagocyte system*** (blood monocytes and resident tissue macrophages) and ***granulocyte system*** (*polymorphonuclear leukocytes* – neutrophils, basophils, eosinophils).

Saliva of healthy individuals steadily contains cellular elements usually moved from gingival crevices and pockets. Approximately 90% of cells within gingival crevice pertain to polymorphonuclear leukocytes, 10% are mononuclear cells. Mononuclear leukocytes encompass 60% of B cells, 20-30% of T cells and for about 10-15% of macrophages.

Granulocytes and macrophages of oral cavity engulf and then destroy the invaded bacterial pathogens. Phagocytes migrate and accumulate within inflammatory focus under the action of versatile groups of *chemokines* both of host or microbial origin (*IL 8* and other cytokines, C3a and C5a complement fragments or *anaphylotoxins*, bacterial peptidoglycan, teichoic acids, and many others).

Microbial killing depends on vast number of bactericidal reactions generated by phagocytes. The most potent microbicidal mechanism is known as ***respiratory burst*** resulting in large amounts of *reactive oxygen intermediates* (*ROI*). The main ROI agents are *superoxide anion* (O_2^-), *hydroxyl radical* (•OH) and *hypochlorite* (OCl^-) that break down biopolymers of bacterial cells within phagosomes. Digestion of bacteria is also supported by numerous antimicrobial peptides (defensins) and hydrolytic enzymes (cathepsins, elastase, lyzozyme, and many others) produced by phagocytes.

In parallel to microbial digestion, macrophages and dendritic cells activate antigenic presentation and enhance secretion of pro-inflammatory cytokines (IL-1, IL-12, IL-18, α-TNF, etc.). This stimulates the differentiation of naive Th0 cells into Th1 generating local inflammatory response in oral cavity.

Inflammatory state can be profoundly aggravated in case of infection spread from primary oral site to regional lymph nodes and further to bloodstream (*systemic inflammatory response*). It is followed by macrophage stimulation with bacterial LPS (or bacterial endotoxin) via its binding to membrane CD14 receptor molecule. This leads to massive overproduction of pro-inflammatory cytokines resulting in fever, hypotension, and if not treated – to multiple organ dysfunction and toxic shock.

Viral infections of oral cavity are controlled by another powerful mechanism of innate immunity – activation of ***natural killers*** or ***NK cells***.

It is a rather small cell population (5-15% of total lymphocyte count), containing great number of azurophilic granules in cytoplasm. These large-size lymphocytes exert the lysis of virus-infected target cells regardless of their antigenic specificity (so-called ***non-immune cytolysis***). In addition, natural killers can destroy some bacteria.

NK cells release a great variety of cytotoxic substances – *perforin*, which resembles in action the membrane attack complex (MAC) of the complement, β-tumor necrosis factor, some special cytolytic enzymes, or *granzymes*, that activate apoptosis. Also they induce cell death activating apoptosis of target cells via CD95-Fas ligand interaction.

Infected cells become sensitive to natural killers owing to impairment of HLA I-class expression on their surface. In normal conditions NK cells bear *killing-inhibiting receptors*, which through interaction with HLA I-class antigens of host cells permanently suppress NK activation. After viral infection of the cell the expression of HLA I-Ag complex alters, and HLA conformation appears to be distorted. This provokes natural killer activation with subsequent lysis of infected cell.

In addition, NK cells were proven to have another type of surface molecules, which directly initiate their killing activity – *killing-activating receptors*.

Overall, non-specific reactions of innate immune response can efficiently eliminate most of microbial invaders. But in situations of progressive oral infections *antigen-specific acquired immunity* should be stimulated.

2.4. The mechanisms of acquired immune response in oral cavity

2.4.1. A brief outline of oral antigen-specific immune response

In general, the reactions of acquired immunity are much more complex and intricate affecting all lineages of immune cells. In oral cavity antigen-specific immune response originates from two main sources.

First, oral pathogens trigger *direct activation of resident immune cells* of salivary glands. Among them are antigen-presenting cells (APC), consisting of dendritic cells (DC) and macrophages, T lymphocytes and B lymphocytes. Microbial antigens undergo natural retrograde flow along salivary ducts. Next they are delivered to immune cells via endocytosis by ductal epithelium.

Second, specific immune response in oral cavity is maintained by continuous *migration of Ag-specific immune cells* to salivary glands from primary sites of antigenic exposure, mainly from *gut-associated lymphoid tissues* or GALT. These cells comprise activated T lymphocytes and specific B cells capable of IgA synthesis. The lymphoid follicules of GALT carry follicule-associated epithelial cells (FAE cells) or *microfold cells* (*M cells*) that capture and transport exogenous antigens from the lumen of small intestine to neighboring dendritic cells for antigen presentation. Afterwards, antigen-stimulated immune cells begin to spread

to various peripheral sites of immune system including oral cavity.

Similar with GALT, expansion of primed immunocompetent cells to oral cavity occurs from NALT, or *nasal mucosa-associated lymphoid tissues* (tonsils and other accumulations of lymphoid follicles within Waldeyer's pharyngeal lymphatic ring).

Taken together, it can be concluded that IgA-producing plasma cells and effector T lymphocytes of oral cavity originate from ***common mucosal immune system***. The latter is the sum of local mucosal lymphoid tissues present in peripheral body compartments and organs.

2.4.2. Thymus-independent antigen-specific immune response

There is a substantial difference of immune response against thymus-dependent and thymus-independent Ags.

Activation of immune system towards thymus-independent pathway is provoked by limited number of antigenic substances restricted by structure and charge.

At first, polymeric complex Ag binds to specific antibody (Ab) receptors of IgM class anchored within cytoplasmic membrane of B cells. This leads to cross-linking of two or more molecules of superficial Ab receptor resulting in next signal transmission and B cell activation. Activated B lymphocytes start transformation into plasma cells that intensively produce specific Ab but of IgM class only. Without T cell help (particularly of IL-4 absence) B cells are unable to switch Ab synthesis from IgM to other Ig classes including IgA thus averting further Ab maturation. Overall, T cell-independent response has essential strong limitations demonstrating rather low grade and specificity.

In contrast, specific response against thymus-dependent Ags initiates multiple immune reactions that profoundly affect host immunity.

2.4.3. T-dependent immune response

T-dependent immune response evolves in three main stages:

1. Antigen processing and presentation

2. Activation and differentiation of T helper cells

3. Activation of effector cells

2.4.3.1. Antigen processing and presentation

Processing and presentation of Ags is the main function of specialized ***antigen-presenting cells*** or ***APC***. Among them are dendritic cells (DCs) of monocytoid and plasmacytoid origin, Langerhans' cells, follicular

dendritic cells, and some others. Macrophages and B lymphocytes are also capable of presenting Ags for T cells.

Once captured by APC (e.g., by different types of DCs, Langerhans' cells, etc.) Ag degrades up to small antigenic peptides within *endosome* by number of proteases (***antigen processing***). Resulting fragments of Ag are next coupled with *HLA II class molecules* inside APC cytoplasm. This interaction requires specific binding of intracellular HLA chains predominantly to antigenic peptide that demonstrate highest affinity to them. Therefore, processed antigenic substances that don't match well any HLA molecule will evade T cell response.

The specific complex (*antigenic peptide - HLA II class molecule*) is transported to APC membrane and next exposed upon it for recognition by Th0 cells (***antigenic presentation***).

Ags of intracellular microbial pathogens (viruses, chlamydiae, rickettsiae and others) are processed directly inside cytoplasm of infected cells within special structure known as *proteasome*. Proteasome is supramolecular cellular complex composed of a number of specific proteases. Resulting antigenic peptides associate with *HLA I class* molecules for further presentation to cytotoxic T cells.

It is generally ascertained that antigen-presenting dendritic cells of various origin play a key role in activation of T cell-dependent reactions. These reactions hinge strongly on *type of initial Ag recognition by APC*.

Ag-presenting cells bear multiple ***pathogen-binding receptors*** (***PBR***) of different types. For instance, high pathogen-binding activity is essential for molecules of ***Toll-like receptor*** (***TLR***) family. More than 10 members of TLR family are known to date. These receptors possess strong binding capacity to ***pathogen-associated molecular patterns*** (***PAMP***) – microbial structural units that are *similar* or *common* for vast groups of pathogens. Other families of receptors (e.g., NOD, RP) are also implicated to primary binding of some groups of Ags.

Hence, antigenic uptake by dendritic cells via ***TLR-4*** activates production of pro-inflammatory cytokines (IL-1, IL-6, IL-12, IL-18, TNF-α). This leads to next transformation of ***Th0 to Th1***, promoting ***cell-mediated reactions and inflammation***.

By contrast, immune activation resulted from Ag binding to ***TLR-2*** on APC membrane triggers secretion of another set of regulatory cytokines (IL-4, IL-10, IL-13, etc.) The latter ensures conversion of ***Th0 into Th2*** lymphocytes thereby promoting ***B cell transformation to plasma cells and antibody secretion*** (see below). This pathway is also stimulated by antigen-presenting B lymphocytes.

2.4.3.2. Activation and differentiation of T helper cells

Principal event that biases immune reactivity towards cellular inflammatory response is the transformation of naive Th0 cells into Th1 cell type whereas conversion of Th0 lymphocytes into Th2 cells activates humoral response and antibody production.

This needs a number of specific stimulatory signals from APC to T helper cells:

a) ***processing of Ag and presentation*** of membrane Ag-HLA complex for recognition by T helper cells;

b) ***expression of costimulatory molecules*** upon APC membranes for additional activation of T helpers;

c) release of ***specific set of cytokines*** that stimulate T helper differentiation.

T helpers recognize complex "Ag-HLA II" by *antigen-specific membrane receptor **TCR***. CD4 and CD3 molecules of Th cells also take part in the reaction making recognition more stable. This delivers *first signal* for T helper activation.

At the same time the expression of costimulatory molecules upon membranes of helper cells is activated. T cells bear specific costimulatory molecule CD28 that binds to its counterpart molecule CD80/CD86 expressed on membrane of APC. This interaction provides *second signal* for activation of T helpers. Without costimulation T cell binding to processed Ags leads not to activation but to suppression and apoptosis of reacting cell clone. It results in *anergy* (or *cell unresponsiveness*) in concern to recognized Ag.

If antigen-specific B-lymphocyte serves as antigen-presenting cell it expresses another costimulatory molecules for activation. Of most value here is CD40 receptor that reacts with specific CD40 ligand (CD40L or CD152 molecule) upon Th0 membrane. This elicits transformation of Th0 into Th2 cells stimulating humoral response with antibody production.

Third signal for differentiation of Th0 into Th1 or Th2 cells ensues from alternative cytokine activation of T helper cells. Type of cytokine secretion substantially depends on primary Ag recognition by APC via specialized pattern-recognizing receptors. As mentioned above, stimulation of APC by TLR-4 activates production of pro-inflammatory cytokines (IL-1, IL-12, IL-18, TNF-α, etc.) thereby causing Th1 differentiation. In contrast, Ag binding to TLR-2 triggers the release of IL-4, IL-10, IL-13 that promotes Th0 maturation into Th2 cell type.

Th1 and Th2 are characterized by opposite type of cytokine secretion. In particular, major cytokines, produced by Th1 cells are γ-interferon, IL-2

and β-TNF, whereas cytokine array of Th2 encompasses IL-4, IL-6, IL-10, IL-13, and some others. Th1/Th2 activity re-routes next immune reactions into ***cell-mediated inflammatory response*** or towards B cell stimulation and antibody synthesis (***humoral response***).

2.4.3.3. Activation of effector cells

Multiple effector mechanisms ensuring elimination of invaded pathogen include cell recruitment to inflammatory focus, stimulation of phagocytosis and antigenic presentation, cytotoxic action of T killers and NK cells, conversion of B cells into plasma cells with secretion of antibodies of various Ig classes.

When differentiated, *Th1 produce gamma-interferon* that activates macrophages and dendritic cells. They greatly enhance production of pro-inflammatory cytokines IL-1, IL-12, IL-18, alpha-TNF thus augmenting further Th0 differentiation into Th1. Furthermore, these cytokines arrest Th2 maturation. Progression of cell-mediated reactions results in *delayed type of hypersensitivity* with ***granulomatous inflammation***.

And vice versa, ***Th2 cells*** produce a broad spectrum of interleukins (*IL-4*, IL-6, IL-10, IL-13). Activation by ***IL-4*** and costimulation of B cells via CD40L of Th2 triggers *blast transformation of B lymphocytes into plasma cells*. This results in antibody affinity maturation and secretion (***humoral immune response***).

B cell *isotypic switching* of immunoglobulin classes from primary IgM next to IgG and (that is the most worthy for oral cavity) to IgA is unlikely without IL-4 stimulation. Newly generated highly specific Abs block Ags, opsonize Ags for phagocytes, activate complement pathways thereby eliminating invaded microbial agents.

In case of immune response against viruses, intracellular bacteria (chlamydia, rickettsiae, etc.) or tumor cells another type of Ag elimination occurs.

Mechanisms of *immune cytolysis* by *T cytotoxic* (Tc) cells include:

(1) target cell recognition and activation of Tc lymphocytes via HLA I-Ag complex expressed by infected or malignant cell;

(2) secretion of killing molecules by Tc cells with subsequent target lysis.

Literally all nucleated cells are capable of presenting Ag for ***T-cytotoxic cells*** (Tc or *T killers*). When infected, the host cell expresses antigenic peptides of invaded pathogens in complex with HLA I class molecules (HLA-A, HLA-B, or HLA-C) upon cytoplasmic

membrane. Complex "Ag-HLA I" is recognized by cytotoxic Tc bearing specific TCRs. This binding is supported by CD8 and CD3 molecules. Activation of Tc needs also additional costimulatory molecules and cytokine stimulation. Once activated, Tcs render cytotoxic activity against infected or tumor cells.

Tc release vast number of cytotoxic factors that eventually cause target lysis. For instance, ***perforin*** is highly active cytotoxic protein produced by Tc. It has cell lytic activity similar with membrane attacking complex of complement. Once embedded into target cell membrane, perforin acts as pore-forming toxin that creates channel within membrane. This leads to osmotic lysis of target cell.

Besides secretion of cytotoxic molecules Tcs demonstrate high capacity for stimulation of ***cell apoptosis***. They start to express large amounts of *FasL* that binds to its counterpart apoptosis receptor CD95 Fas/Apo upon the membranes of affected cells. Under specific binding the target cell undergoes apoptosis.

Similarly, Tcs produce a group of cytotoxic enzymes or *granzymes* that also activate apoptosis pathway.

Finally, Tcs activate secretion of gamma-interferon that arrests viral replication and stimulates antiviral and anti-tumor activity of NK cells.

2.4.4. Natural inhibition of immune response

After peak of high intensity immune reactions gradually fade. Clonal expansion of immunocytes results in formation of long-living memory T- and B cells that come into dormant state until next antigenic challenge. This damps overexuberant or detrimental immune reactivity preventing autoimmunity reactions.

There are numerous versatile mechanisms of natural inhibition and restriction of immune response.

Normally all secreted cytokines are short-living substances. They render discernible activity only within limited space affecting the cell itself and neighboring cells. This prevents undesirable immune expansion.

Furthermore, multiplex interrelationships within cytokine network are characterized by reciprocal suppression of their actions. For instance, IL-4 precludes Th1 differentiation and next cell-mediated response, whereas IL-12 inhibits Th2 thus down-regulating humoral immunity; IL-10 blocks literally all pro-inflammatory cytokines; potent inhibitory activity is proven for TGF-beta (transforming growth factor beta) cytokine.

In addition, cell activation arrests after change of superficial

costimulatory molecules. While CD80/86 promotes Th differentiation via CD28, later substitution of CD80/86 with CD152 inhibits further proliferation of Ths.

And finally, many of activated cell subsets (e.g., plasma cells) raise expression of apoptosis receptor CD95. It triggers their programmed death, for example, after interaction with regulatory T lymphocytes, bearing specific CD95L.

It has been established quite recently that group of ***regulatory T cells*** includes diverse T cell populations endowed with *powerful suppressive function.*

Number of adaptive regulatory T cells is activated directly after antigenic challenge. Among them are CD4(+) Tr1 lymphocytes, producing IL-10, Th3 cells, secreting TGF-beta, and some others.

Another substantial part of T lymphocytes (more than 3% of total T cell count) is primarily differentiated as ***regulatory suppressor cells***. They demonstrate striking inhibitory capacity contributing to immune reactions as natural regulatory T cells. Molecular markers of this cell type are CD4 and CD25 that are co-expressed together upon cell membranes.

Unlike other lymphocytes, natural regulatory T cells contain active form of specific transcriptional factor Foxp3. It is encoded by X chromosome foxp3 gene H – a special molecular label for this cell subset.

During recognition of Ags presented by DCs natural regulatory T cells expose inhibitory molecule CTLA-4 (CD-152). This leads to silencing of cell activation and prevents effective co-stimulation of other T cells by APCs. Furthermore, natural regulatory lymphocytes produce large amounts of suppressive cytokines ***TGF-beta*** and ***IL-10*** that avert proliferation of reactive cell populations.

Another way of immune control is realized by ***idiotypic network regulation***. Any antigen that challenges immune system elicits polyclonal immune response, i.e., stimulates production of specific Abs and TCRs by various cell clones. They display similar but not identical specificity and affinity. It means that generated Abs and receptors have minor structural differences in their antigen-binding sites. Therefore, active sites of newly appeared Abs and receptors would bear unique *antigenic determinants* (named *idiotopes*) that specify only one certain clone of immune cells. Total number of idiotopes carried by single molecule of antibody was termed ***idiotype***.

First generation of Abs arisen against specific Ag during immune response was named *idiotypic* (i.e. bearing idiotype). Once appeared,

idiotypic Abs start to trigger second generation of Abs against their own active sites (so-called ***anti-idiotypic Abs***). The latter inhibit overexpression of primary idiotypic Abs, receptors and cell clones.

All these extremely versatile and powerful reactions of immune and non-immune protection can efficiently block the activity of oral pathogens. Nevertheless, in case of intensive accumulation of microbial agents with noticeable virulence, especially followed by local or systemic immune deficiency, the defense mechanisms of oral cavity may lose their functions resulting in the emergence of infectious pathology of oral cavity.

III. INFECTION-ASSOCIATED DISEASES OF ORAL CAVITY

3.1. Brief outline of teeth and mouth pathology of infectious origin

Now it is generally ascertained that caries, pulpitis, or periodontal diseases are the ailments *essentially related with infection.* They are caused by microbial pathogens has been found in dental plaque.

There are two alternative albeit complementary assumptions concerning the role of dental plaque microflora in pathogenesis of oral disorders.

One of these hypotheses (the hypothesis of *"specific dental plaque"*) presumes the active participation of only limited (or "*specific*") number of bacterial species in the emergence of teeth and mouse pathology.

And on the contrary, certain considerations are made in favor of *"nonspecific plaque hypothesis"*, where most of oral diseases are generated by common *non-specific* deteriorating activities of total dental microbial mass despite their species origin.

To date the first hypothesis has got more confirmations indicating the prevalence of definite bacterial representatives in carious or periodontal lesions. Nonetheless, violation of oral hygienic measures results in rapid growth of bulk microbial biomass thereby elevating the risk of emergence of dental pathology. All this emphasizes the deleterious role of any shift in balance of normal oral microbiota.

But further progression of oral diseases inevitably leads to selection of the limited number of dental and periodontal pathogenic species responsible for basic disease course.

All infections of oral cavity are divided according to affected anatomical region.

Among them are ***dental*** (*caries* and *pulpitis*), ***periodontal*** (all forms of periodontitis) and ***gum diseases*** (*gingivitis*); infections of oral mucosa (*stomatitis*) and salivary glands; suppurative infections of neck and orofacial area (facial bones periostitis and osteomyelitis, sinusitis, lymphadenitis, soft tissue infections of neck and face). These primary infections may spread from initial sites resulting in ***odontogenic*** life-threatening regional or systemic disorders and complications (retropharyngeal, mediastinal or intracranial abscesses or phlegmons; and in exceptional cases, sepsis and septic shock).

Besides mentioned above, in some cases various ***non-odontogenic*** diseases of neck and facial area can arise. They comprise a number of

purulent infections – folliculitis, furuncles (boils) and carbuncles, lymphadenitis, erysipelas, secondary hematogenous osteomyelitis and others.

And finally, orofacial area is commonly affected in patients suffering from the variety of ***specific infectious diseases*** (actinomycosis, tuberculosis, syphilis, diphtheria and many others).

3.2. Pathogenesis of oral infections: several common steps

Infection-associated pathology of oral cavity commences from ***microbial adherence*** to dental and/or mucosal tissues resulting in *dental plaque formation* (microbial *biofilm*). Tight attachment of bacteria to host cells ensues from selective binding of multiple microbial *adhesins* to *membrane cellular receptors* of variable specificity.

Numerous groups of ***bacterial adhesins*** comprise cell wall polysaccharides, teichoic acids, certain bacterial enzymes, e.g., glycosyltransferases, polysaccharide-binding proteins or *lectins* and many others. Their counterparts – receptors of cell membranes – belong to exuberant groups of surface molecules such as mucins, lectins, integrins, members of immunoglobulin superfamily receptors, prolin-containing proteins, various glycoproteins, antibodies together with the considerable amounts of membrane-absorbed bacterial components (glycosyltransferases, glucans, etc.) In substantial number of cases this firm binding demonstrates moderate or low specificity. For instance, bacterial lipoteichoic acids bind to all negatively charged membranes by means of calcium ions.

Nevertheless, selective ***colonization*** of bacteria upon oral epithelium is promoted by specific interactions of microbial pathogens with host cells. In fact, *Actinomyces naeslundii* binds to superficial cell antigens by Ist type fimbria; *S. mutans* reacts with host prolin-enriched proteins, *S. gordonii* – with oral amylase, *S. sanguis* interacts with sialyl-containing oligosaccharides of MG2 mucins.

Moreover, the bacteria dramatically enhance primary oral colonization making tight cross-linkages between attached microbial cells.

The process of bacterial cells cross-binding is termed as ***coaggregation***. The most common is *intergeneric coaggregation* that involves bacterial species from various genera. *Intrageneric coaggregation* occurs more seldom albeit it is typical for oral viridance streptococci. In latter case coaggregation is mediated by lectin binding.

Bacterial coaggregation entails the formation of microbial biofilm and dental plaque.

Synthesis of exopolysaccharides (glucans) from sucrose- or glucose-containing nutrients by *Streptococcus mutans* plays pivotal role in coaggregation of dental pathogens. Streptococcal glycosyltransferases produce polymeric glucans attached to microbial envelope. This elicits tight binding of streptococci to dental surface as well as to other bacterial cells via numerous microbial lectins.

Furthermore, the oral pathogens can directly impair host immune response making havoc of host defense factors. For instance, a great number of pathogenic bacterial species (e.g., *S. sanguis*, *S. oralis*, *S. mitis*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Capnocytophaga spp.* and others) are capable of producing high-rate IgA-proteases that actively destroy salivary sIgAs. This substantially lowers the grade of oral cavity protection.

Overall, under poor situation with oral hygiene, malnutrition or starvation conditions, or host endocrine and immune disorders bacterial colonization of oral cavity progresses rapidly. It biases the established equilibrium among normal oral microbiota towards the prevalence of pathogenic bacterial species. This in turn leads to preponderance of aggressive influences over protective ones within oral cavity. Finally the defense barriers can be broken down, and deleterious activities of microbial and non-microbial origin simulate the progression of certain oral disorders – e.g., dental caries, pulpitis or periodontitis.

3.3. Dental caries

Caries is *dental disease of bacterial origin* affecting all of *dental hard tissues* (enamel, dentin and cementum) that is followed by demineralization and progressive decay of tooth structure *resulting in cavity formation.*

For about 2.5 billion people (approximately one third of global population) demonstrate dental caries of permanent teeth.

Usually caries emerges as local bacterial process. It starts from dental plaque expansion. Common tooth sites affected by caries are coronal surfaces, especially their fissures and pits. The disease can arise also on bare parts of dental roots in case of gingival recession.

General scheme of *pathogenesis of dental caries* looks as follows: demineralization (primarily, decalcification) of tooth hard tissues is caused by accumulation of organic acids in the offing of dental surface.

The rise of concentration of organic acids in oral cavity ensues from fermentation of food-derived carbohydrates (e.g., sucrose) by certain ***acidogenic species*** of indigenous oral microflora. The shift of pH towards acidity creates the conditions for selective propagation of so-called ***aciduric bacteria*** or capable of tolerating acids. These bacteria intensify acid production.

Organic acids (lactate and others) dissolve dental tissues resulting in tooth decalcification. A crucial pH level for start of tooth demineralization should be equal or less than 5.0-5.5.

A vast number of experiments and clinical observations confirmed the major role of viridance streptococci (and first of all – of species *S. mutans* and *S. sobrinus*) in caries emergence and progression. Acidification of microenvironment spurs the next growth of lactobacilli in primary lesions.

These two bacterial groups can rapidly metabolize carbohydrates predominantly with lactic acid byproducts. First steps of the disease are related with *S. mutans* and *S. sobrinus* activity. Lactobacilli grow more slowly being concurrent with clinical signs of caries. Thus streptococci are *cariogenic bacteria* that initiate caries of dental tissues whereas lactobacilli are more responsible for disease progression.

Pathogenicity of *S. mutans* is maintained by its high adhesive capacity to dental enamel that stimulates dental plaque growth. *S. mutans* produces the enzymes glycosyltransferase and fructosyltransferase. They polymerize food-derived glucose and fructose into insoluble polysaccharides glucan and fructan. Glucan is the leading substance promoting adherence and coaggregation of *S. mutans* and other bacteria with reinforcement of dental plaque structure. For example, actinomycetes reside in dental biofilm via binding with their fimbria to biofilm glucans.

At neutral pH dental plaque harbors relatively low amounts of *S. mutans* and lactobacilli. By contrast, abundant consumption of sugar-containing nutrients results in their active microbial fermentation yielding lactic acid as the main byproduct that lowers dental plaque pH. This in turn dampens the growth of many resident bacterial species, such as *S. mitis, S. oralis, S. sanguis*, but accelerates propagation of *S. mutans* and lactobacilli. It biases local tooth metabolism to progressive demineralization. If oral defense factors (mainly, salivary flow and bicarbonate buffer) are unable to neutralize detrimental activity of pathogenic microflora the dental caries ultimately appears.

Meanwhile, the list of cariogenic microbial pathogens is not limited with the above mentioned bacterial species. In parallel with streptococci and lactobacilli the carious lesion confines broad spectrum of indigenous dental microflora. It was established quiet recently by methods of molecular genetic analysis (polymerase chain reaction or PCR, ribotyping, DNA and RNA microarray hybridization analysis) that many other bacterial species tamper with dental caries

Among them are *Actinomyces gerencseriae*, *Bifidobacterium spp.*, *S. salivarius*, *S. constellatus*, *S. parasanguinis* and others. In particular, *Actinomyces gerencseriae* is supposed to play a role in caries initiation, whereas the activity of bifidobacteria accounts for profound caries. On the contrary, domination of *S. sanguis* in dental plaque slows down the disease progression. Thus, dental caries results from deranging of complex multiple interplays normally established within oral microbiota.

If arisen but not treated, ***dental caries*** passes through several consecutive ***steps***:

(1) initial caries;
(2) superficial caries;
(3) moderate caries;
(4) deep caries
(5) deep complicated caries.

Initial caries appears as primarily white, then yellowish and later brownish spot. It is characterized by local tooth demineralization without visible structural changes. Initial caries is reversible if active mouth hygiene and fluoridation will be done.

Superficial caries affects enamel demonstrating wedge-shaped enamel defects but without dentin involvement.

Moderate caries corresponds to marked dentin damage.

Deep caries is followed by deep dentin penetration in closest vicinity to the pulp.

Deep complicated caries results in opening of the pulp cavity with pulpitis, periodontitis or abscess formation.

This division is generally consistent with *WHO classification* that includes four grade scale:

D1. clinically detectable enamel lesions with intact (non-cavitated) surfaces;

D2. clinically detectable cavities limited to enamel;

D3. clinically detectable cavities in dentin;

D4. lesions extending into the pulp.

3.4. Treatment and prophylaxis of caries

The treatment of dental caries depends on its stage. Initial caries and non-cavitated lesions don't need operative treatment. As the initial caries is reversible, enhanced oral hygiene favors remineralization. Topical fluoride therapy, e.g., fluoride varnish, demonstrates high preventive and treatment efficacy.

Cavitation requires restorative dentistry with operative interventions. All of the destroyed tissues should be removed with subsequent cavity filling.

Caries prophylaxis is primarily based on adequate oral hygiene and proper dietary recommendations with limited consumption of food sugars (e.g., "table sugar") and sticky foods like candies. A proper dental hygiene presumes regular teeth cleaning with toothbrushes and interdental brushes, flossing, the use of chewing gums with xylitol, etc.

Fluoride- and biocide-containing toothpastes evidently foster caries prophylaxis: oral antiseptics inhibit the growth of cariogenic microflora; fluorides stimulate calcification of dental hard tissues. Usage of dental sealants isolate teeth surface from aggressive external influences.

Specific prophylaxis of caries by vaccination still remains the subject of experimental medical design. Based on genetically modified strains of *S. mutans* or lactobacilli several experimental anti-caries vaccines undergo clinical trials now but their preventive efficacy requires further unbiased confirmation.

The results of caries prevention by topical applications of soluble antigens derived from cariogenic bacteria also remain controversial and need further elucidation.

3.5. Pulpitis

Pulpitis or *inflammation of dental pulp* arises predominantly as *complication of deep caries*, where profound dentin decay provokes pulp exposure to aggressive activity of microbial and other inflammatory factors.

Emergence of pulpitis is stimulated also by dental traumas, chemical irritation of pulp with dental restorative materials (e.g., sodium fluoride or phosphoric acid), surgical treatment of periodontal diseases or other medical interventions.

Nonetheless, it is obvious that the major role in etiology of pulpitis should be reserved for infectious agents. A multitude of oral pathogens may participate in pulpitis. Most common causative agents are numerous species of α-hemolytic streptococci, representatives of gram-negative non-sporeforming anaerobic rods (bacteroids, fusobacteria, porphyromonads, prevotellas) and gram-positive anaerobic cocci (or *GPAC*) – peptococci and peptostreptococci; actinomycetes and lactobacilli.

From carious cavity the bacteria enter the primarily sterile pulp through dentinal canaliculi, in some cases – by apical channel of dental roots. Also pulpitis might be borne from extradental infectious sites, such as infected gingival pockets, or as the result of sinusitis or orofacial osteomyelitis. The spread of hematogenous infection into the pulp is seldom observed.

Acute pulpitis is characterized by sudden onset and rapidly progressive inflammation with edema and sharp pain. It impairs dental blood supply.

Reactive inflammatory response in pulp is promoted by activity of innate immune cells against microbial pathogens. They comprise neutrophils, dendritic cells, T cells, natural killer cells, macrophages, odontoblasts. All these cells produce exuberant amounts of antimicrobial peptides, cytokines, chemokines and enzymes.

In several hours active purulent exudation may lead to periodontal inflammatory infiltrations or abscesses. If not treated, acute pulpitis exerts pulp necrosis, sometimes complicated with apical periodontitis; in case of modest activity it resolves into chronic process.

The *treatment* of reversible pulpitis foremost implies entire and high-quality treatment of caries. Removal of hard tissue decay and cavity restoration dampens inflammation and causes pulp healing.

Irreversible pulpitis with non-vital pulp requires endodontical treatment followed by removal of irreversibly damaged pulp.

Antimicrobial therapy is applied in cases of infection spread from pulp into surrounding tissues resulting in periodontitis, periostitis, regional lymphadenitis, or other complications. Beta-lactam antibiotics, doxycycline or anti-anaerobic drugs (metronidazole, clindamycin) can be administered.

Prophylaxis of pulpitis is non-specific. It depends on adequate dental care.

3.6. Periodontal diseases

Periodontal pathology is the group of inflammatory diseases of infectious origin affecting any of tooth-supporting tissues (alveolar bone, periodontal ligament, cementum and gingiva).

Periodontal diseases are induced by deleterious activity of infectious agents concentrated in dental plaque. Poorly manifested, microbial pathogens stimulate local inflammatory responses that eventually lead to tissue atrophy with progressive collagen loss from tooth-supporting structures.

Inflammatory periodontal disorders are divided into 2 main categories: *gingivites* and *periodontites*.

More than 50% of adult population have gingivitis and above 30% suffer from periodontitis.

3.6.1. Gingivitis

Gingivitis is the *inflammatory gum disease*. It is characterized by superficial inflammation affecting gums only. In these cases dental hard tissues and dental ligament still remain intact; and the depth of periodontal pockets doesn't exceed 3 mm.

Gingivitis begins from dental plaques expansion over gingival margin. Normally, progression of dental plaque is strictly limited by adequate dental hygiene that removes the most of oral pathogens. As the result, only low amounts of facultatively anaerobic gram-positive bacteria remain within gingival crevice. But in case of gingival inflammation total number of microbial cells increases rapidly up to 10-20 times from initial. It is followed by active preponderance of anaerobic gram-negative bacterial species amongst crevicular microbiota.

Non-specific (*plaque-induced*) gingivitis is the disease of evident polymicrobial nature. *S. sanguis*, *S. mitis*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Actinomyces naeslundii genospecies 2* (formerly known as *A. viscosus*), bacterial members from genera *Veillonella*, *Wolinella*, *Capnocytophaga* are commonly isolated.

By contrast, *non-plaque-accociated gingival lesions* comprise specific bacterial, fungal and viral gingivites, caused by certain microbial pathogens. These infectious agents are able to exert the direct damage of gum and oral mucosa in the course of primary infection.

Non-infectious secondary gingivites may follow systemic autoimmune or genetic disorders; or traumatic lesions.

Beyond the vast number of non-specific (or plaque-induced) microbial gingivites there is a special form of bacterial disease known as ***acute necrotizing ulcerative gingivitis*** or ***ANUG***.

It affects predominantly young persons, demonstrating sharp onset and severe pain due to *necrosis of interdental papilla* (gingival parts between the teeth). This acute gingival damage was primarily described by French physician H. Vincent and thereafter named as "Vincent's angina" or "Vincent's disease".

During the years of World War I the disorder was known as "trench mouth", afflicting predominantly military staff. However, it may occur in any person in conditions of starvation or malnutrition and under stress.

H. Vincent ascribed the etiology of the disease to mixed spirochetal and fusobacterial infection mainly due to permanent detection of this agents within specific oral lesions. The presence of large spirochetes with irregular coils carrying more than 20 fibrils was commonly found in clinical specimens taken from these patients.

Now it is generally ascertained that acute necrotizing ulcerative gingivitis arises from complex polymicrobial infection, where major role belongs to oral spirochetes and the members of gram-negative anaerobic *Prevotella intermedia* species.

The *treatment of microbial gingivitis* includes the administration of oxidizing agents (hydrogen peroxide or iodine) and the use of antimicrobial drugs, affecting anaerobic bacteria, such as metronidazole. Rinsing of oral cavity with solutions of oxidants (e.g., hydrogen peroxide) prevents the emergence of acute necrotizing ulcerative gingivitis. Overall, adequate prophylaxis and treatment ensures favorable prognosis of these disorders.

3.6.2. Periodontitis

Periodontitis is the inflammatory polymicrobial infectious disease affecting supportive dental tissues that if not treated, leads to tissue attrition with progressive collagen degradation, alveolar bone resorption and eventual tooth destabilization or loss.

3.6.2.1. Pathogenesis of periodontitis

Pathogenesis of periodontal diseases is a complex multifactorial process comprising dental plaque overgrowth, exuberant accumulation of microbial wastes and virulence factors ultimately resulting in local progressive inflammatory response.

In the course of disease the gingival crevice deepens over 3 mm and transfigures into periodontal pocket gradually expanding from 4 to 10-12 mm and even more. Every pocket may contain 10^7-10^9 of microbial cells. Detrimental activity of microbial pathogens harbored in the pocket accounts for disease progression and tissue destruction.

The composition of local microbial communities changes grossly following the development of periodontitis.

As early as in 1998 S. Socransky with coworkers proposed to divide the members of oral microbiota into *separate groups* that *correspond to healthy or pathological conditions* found within oral cavity.

Each group harbors a number of related microbial species that are commonly isolated at certain steps of dental plaque growth or, by contrast, when pathology arises. However, there are striking dissimilarities observed between the groups. Therefore, every group reflect unique colonization pattern essential for various microbial communities.

By S. Socransky, different "***colors***" were assigned to these microbial clusters, named as "***complexes***".

It was pointed out that "*purple*", "*cyan*" "*yellow*" and "*green*" complexes comprise early first colonizers of the tooth surface especially of its subgingival sulcus. Thus, the members of these complexes were primarily ascertained as the residents of healthy gums.

"***Purple*** or *magenta* ***complex***" is closely associated with healthy gingival state and includes species *Veillonella parvula* and *Actinomyces odontolyticus*.

"*Yellow* complex" encompasses a number of streptococci (*S. sanguis*, *S. mitis*, *S. gordonii* and *S. intermedius*), "*cyan* complex" – numerous actinomycetes.

The bacteria from purple and yellow complexes are regarded as *protective microbial agents* demonstrating antagonistic activities against pathogenic microflora.

"*Green* complex" was found to contain diverse microbial agents such as *Eikenella corrodens*, *Aggregatibacter actinomycetemcomitans serotype a*, *Campylobacter concisus*, *Capnocytophaga spp.* It has been shown after close scrutiny that species of green complex can actively participate in progression of serious dental pathology, e.g., periodontites with tissue destruction.

Finally, the bacteria from *red* and *orange complexes* demonstrate intimate association with oral pathological conditions.

Three members of ***red complex*** – gram-negative obligate anaerobes *Porphyromonas gingivalis*, *Tannerella forsythia* (former *Bacteroides*

forsythus), and *Treponema denticola* are the pivotal periodontal pathogens commonly isolated in ***chronic periodontitis*** with deep pockets and gingival recession.

The ***orange complex*** embraces the variety of anaerobic pathogens *Prevotella intermedia* and *Prevotella nigrescens*; *Streptococcus constellatus*, *Eubacterium nodatum*, *Peptostreptococcus micros*, several species from genera *Fusobacterium* (*F. nucleatum*, *F. periodonticum*), and *Campylobacter* (e.g., *C. rectus*).

The bacteria of orange complex are associated with ***gingivitis*** and gingival bleeding. They are tightly related with red complex members demonstrating mutual pathogenesis.

Three other pathogenic species, namely *A. actinomycetemcomitans serotype b*, *Selenomonas noxia* and *Actinomyces naeslundii genospecies 2* (formerly *A. viscosus*) don't' pertain to any outlined complex but intensively impact on progression of dental pathology as well.

Division of bacteria into pathogenicity groups or "complexes" strongly correlates with clinical situation in periodontal diseases. These disorders are proven to be ***inflammatory*** injuries of ***polymicrobial origin.***

Normal microbiota of subgingival plaque commonly harbors facultatively anaerobic gram-positive bacteria (mainly, streptococci), actinomycetes and anaerobic gram-negative veillonellas (*purple* and *yellow complexes*), but only 5% of spirochetes or anaerobic motile rods.

In case of irregular and poor oral hygiene the expansion of dental plaque accelerates secretion of crevicular fluid and stimulates local inflammatory response. If not recovered, microbial metabolism and inflammation turns down crevicular redox potential thereby affording the growth of anaerobic bacteria. Most of them release powerful virulence factors with proinflammatory (e.g., bacterial LPS), enzymatic (collagenase, elastase, hyaluronidase, etc.) and toxic activities. Gram-negative anaerobes from genera *Porphyromonas*, *Prevotella*, *Fusobacterium*, *Tannerella*, *Aggregatibacter*, *Capnocytophaga*, *Wolinella*, and *Treponema* substantially worsen the local periodontal status.

Tissue matrix metalloproteinases and bacterial hydrolytic enzymes destroy supportive dental surroundings with marked collagen degradation. Persistent inflammation converts into chronic periodontal disease. The latter results in dental pocket excavation, gingival recession and final tooth destabilization.

Overall, in course of chronic periodontitis naturally present bacteria of "purple" and "yellow" complexes are gradually substituted by periodontal pathogens of "red" and "orange" microbial groups. In these conditions

gram-negative bacteria comprise 75% of total cells, and what's more, 90% pertain to strict anaerobes.

3.6.2.2. Clinical variations of periodontitis

There are several kinds of periodontitis that are different in clinical course.

Among them are *chronic periodontitis*, *aggressive periodontitis*, periodontitis as a manifestation of systemic diseases; periodontites, associated with genetic or hematological disorders. All of these forms can be *localized*, *generalized* or *refractory* (see Table 2).

Table 2.
Classification of periodontal diseases
from The American Academy of Periodontology, 1999

I. Chronic periodontitis	II. Aggressive periodontitis	III. Periodontitis as a manifestation of systemic disease	IV. Necrotizing periodontal disease
Localized Generalized Refractory	Localized Generalized Refractory	Associated with hematological disorders Associated with genetic disorders	

Also periodontites are divided into the disorders affecting individuals of 35 years or younger, and periodontites of adults (the disease in persons over 35).

At last, there are special clinical forms of periodontitis, for instance, *local juvenile periodontitis* and necrotizing periodontal disease.

3.6.2.3. Local juvenile periodontitis

Local juvenile periodontitis predominantly affects teenagers with annual morbidity rate from 1 to 5 in 1,000 of population.

In absence of treatment, rapid degenerative lesions after the disease emergence lead to ultimate dental loss.

In spite of its severity, the low volume of dental plaque is characteristic for this pathology. It damages mainly molar and incisor teeth without extensive plaque or calculus formation.

Unlike other kinds of periodontites, the disease has narrow range of microbial species in its etiology. In most of cases microaerophilic bacteria *Aggregatibacter actinomycetemcomitans* are isolated from periodontal lesions. They are cultured on malachite green-bacitracin selective medium.

This periodontal pathogen produces potent virulence factors, such as *LPS* with proinflammatory activity and highly active *leukotoxin*.

Leukotoxin suppresses the actions of neutrophils thus ensuring microbial penetration into nethermost tissues. In some cases the infection may spread further to the bloodstream. Generalization of infectious process triggers both local and systemic immune responses.

Manifested severe forms of the disease are supposed to evolve in children, which carry genetic defects in neutrophil chemotaxis. Protracted course of local juvenile periodontitis allows other microbial species (e.g., anaerobic bacteria) to participate in disease pathogenesis thereby worsening its prognosis. Nevertheless, administration of broad spectrum antibiotics (doxycycline or metronidazole) successfully interrupts the infectious process.

3.6.2.4. Periodontitis of young individuals (early-onset periodontitis, aggressive periodontitis)

This form of the disease afflicts the young persons below the age of 35. It may affect from 0.5 to 2% or even more of children and young people. The most of disease cases are followed by dental plaque broadening and calculus formation. The infection moves to periodontal tissues and actively propagates there. In case of aggressive disease course eventual dental loss is possible even before the age of 20.

It was long-time supposed that a major role in etiology of early-onset aggressive periodontitis belongs to periodontal pathogen *A. actinomycetemcomitans*. However, it has been found by now that the disease emergence and progression is not primarily related with microaerophilic bacteria but depends on limited number of obligate anaerobic bacterial species *Treponema denticola*, *Prevotella intermedia*, *Tannerella forsythia*, *Porphyromonas gingivalis* and some others.

The prophylaxis and treatment of this ailment is generally similar to above-mentioned clinical forms of the disease.

3.6.2.5. Adult periodontitis (or chronic periodontitis)

It is one of the most common infectious diseases affecting adult population. Usually it demonstrates slowly progressive course with multiple dim exacerbations. It is characterized by low-grade or moderate

inflammation resulting in degenerative lesions of periodontal tissues and tissue atrophy.

The disease is evidently of *polymicrobial origin* with high *prevalence of anaerobic bacteria*. The culturability of most of this species is very low; that's why they can be discovered only by methods of molecular genetic analysis (polymerase chain reaction or PCR and DNA hybridization).

By lowering of redox potential in periodontal pocket, a selective growth of bacteria from red and orange complexes accompanied by numerous microaerophilic pathogens is stimulated. The association between microaerophil species *A actinomycetemcomitans, Wolinella recta, Eikenella corrodens* and obligate anaerobes *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Prevotella intermedia, Fusobacterium nucleatum* and some others is typically observed.

Peri-implantitis, the disease resembling periodontitis, but affecting dental implant surroundings, demonstrate similar behavior. Failing implants and residual teeth can be the subject of bacterial attack.

Despite the striking variability of microbial communities residing in dental pockets, the *therapy of chronic periodontites* should be oriented on prevention or restrain of only limited number of the most active periodontal pathogens. As they are predominantly anaerobic, the administered drugs must impact mainly this group of bacteria. Most effective are *metronidazole, clindamycin, doxycycline* or beta-lactams. They can be used locally by applications of polymeric antibiotic-containing films into dental pockets or by systemic administration in severe cases.

Drug therapy should be only the constitutive part of complex versatile treatment of chronic periodontitis. It comprises also the debridement procedures (scaling and root planning) and, if needs, dental surgery. Taken together, these procedures terminate the disease progression and prevent tooth loss.

3.7. Periostitis and osteomyelitis of mandibular and maxillofacial region

Jaw ***periostitis*** is the inflammation of periosteum of corresponding bone.

In majority of cases it ensues from complicated acute periodontitis or as the result of exacerbation of chronic periodontal disease. More seldom it arises from periapical abscess, suppurative radical or follicular cysts,

wounds after dental extraction, etc.

Osteomyelitis is the inflammatory process affecting bones and/or bone marrow.

Osteomyelites are divided into *suppurative* or infectious (the most common) and *non-suppurative* (e.g., after aseptic traumatic injury). They are also classified into *acute* and *chronic* (with duration more than 1 month).

In dentistry osteomyelites of orofacial area can be odontogenic (from dental infection) and non-odontogenic (hematogenous, post-traumatic and others).

The most commonly isolated microbial pathogens that cause periostitis and osteomyelitis are *S. aureus* (more than 80% of cases in adults), group A streptococci, *Enterobacter* species; and *H. influenzae* in children.

Suppurative post-traumatic osteomyelitis can be provoked by *S. aureus*, *Enterobacteriaceae* members or *P. aeruginosa*.

Complex treatment of periostitis and osteomyelitis encompasses adequate surgery of infectious sites and antimicrobial treatment with antiseptics and antibiotics. Antiseptics (e.g., *chlorhexidine*) are applied locally; antibiotics should be administered according to the results of laboratory testing of microbial antibiotic resistance. The most commonly used drugs for antimicrobial treatment in these cases are beta-lactams in combinations with β-lactamase inhibitors, e.g., *amoxicillin-clavulanic acid*; in case of *P. aeruginosa* infections carbapenems, piperacillin/tazobactam or ceftazidime are used.

3.8. Odontogenic maxillary sinusitis

Acute or chronic sinusitis is relatively rarely caused by tooth infection.

Nevertheless, odontogenic maxillary sinusitis makes up to 20% of total cases of maxillary sinusitis.

The most common agents of odontogenic maxillary sinusitis are *S. pneumoniae*, *H. influenzae,* or *M. catarrhalis*, sometimes *S. aureus* or non-sporeforming anaerobic bacteria. Vaccination against *H. influenza* type B (or Hib) substantially reduced the incidence of sinusitis caused by this bacterial pathogen.

In most cases of acute sinusitis antibiotic treatment is not used. If the disease exceeds 10 days of duration, a short 3-7 days course of amoxicillin/clavulanate is recommended.

3.9. Suppurative infections of soft tissues of facial and neck areas

Suppurative odontogenic infection of face and neck soft tissues is rare but highly severe complication of primary infections of oral cavity. It affects muscles, subcutaneous fat, blood vessels, fibrous connective tissue and fascia of orofacial and neck regions.

These injuries are clearly of polymicrobial nature with active participation of streptococci, staphylococci, bacteroids, fusobacteria, peptostreptocci, in case of hospital infections – *Pseudomonas aeruginosa.*

Once appeared, suppurative infections spread from initial site resulting in *abscess* or *phlegmon* formation.

Tissue ***abscess*** is the localized cavity filled with the pus, bacteria, phagocytes and elements of destroyed tissues (or debris). It is surrounded with *abscess wall* or *capsule* made of inflammatory granulation tissue. Encapsulation acts to hold pus inside and prevents its further spread but the same time it restrains the activity of immune cells.

Phlegmon is the severe purulent inflammation of soft tissues that actively spreads from primary site without tendency to self-limitation. It affects muscles, fascias and adipose tissue resulting in non-capsulated pus accumulation amid muscles and fascial compartments.

Odontogenic phlegmons or abscesses originate from initial infectious sites located in teeth or periodontal lesions, inflamed salivary glands (*sialadenitis*), tonsil crypts, lymph nodes or other structures.

Depending on microbial virulence and immune reactivity, the course of infections might be drastic and aggressive with high fever and intoxication or, by contrast, torpid and faint. Anyway, both situations cannot lead to self-recovery.

This pathology requires active surgical treatment and antibacterial therapy. Patients should be treated with antibiotics according to estimated microbial drug resistance. Beta-lactams in combination with β-lactamase inhibitors, (e.g., *amoxicillin-clavulanate*) can be used as the drugs of first line.

3.10. Facial and neck lymphadenitis

Regional lymphadenitis of neck and facial area usually follows the inflammatory infections of oropharyngeal and facial zones.

Overall, sub-mandibular and neck lymphadenites can arise from *odontogenic* or *non-odontogenic* infections.

Sub-mandibular lymphadenitis and lymphadenitis of anterior and posterior neck surface in children before 4-5 are related mainly with viral infection.

Most common are so-called "*non-specific*" lymphadenites that pose the background for virtually any suppurative lesion of orofacial region.

"Specific" lymphadenites may arise after specific microbial antigen exposure, e.g., BCG vaccination against tuberculosis.

Abscesses of lymph nodes are provoked by secondary bacterial infection spread throughout regional lymphatic system. They are usually caused by typical number of infectious agents, such as streptococci, staphylococci, actinomycetes, gram-negative anaerobic microflora.

Antimicrobial treatment of lymphadenitis is performed as auxiliary measure in complex cure for primary oral infection of bacterial origin.

3.11. Odontogenic bronchial and pulmonary infections

In certain cases dental and periodontal infections can elicit bronchial or pulmonary pathology.

As the oral cavity harbors myriads of microorganisms, some of opportunistic pathogens may cause respiratory diseases (bronchitis or pneumonia) if they reach bronchial or pulmonary tree. Among them are pneumococci, klebsiellas, *S. pyogenes*, mycoplasmas, chlamydiae, bacteroids, several uncommon bacterial agents such as *Moraxella spp.*, *Kingella kingee*, *Acinetobacter baumanii*, or viruses (e.g., herpes virus).

Secondary bacterial pneumonias arise in patients under severe conditions affecting respiratory tract. They occur in case of aspiration of oral or gastric contents in patients with bedridden status or long-time unconscious; in postoperative patients, patients receiving mechanical ventilation, patients with debilitating neurologic disorders; immunocompromised individuals with primary or secondary immune deficiencies, etc.

Secondary pneumonias usually demonstrate *mixed polymicrobial infection*. They are caused by long list of opportunistic bacterial agents (e.g., staphylococci, enterococci, klebsiellas, pseudomonads, enterobacterial pathogens, anaerobic bacteria). In many situations this microflora can be of odontogenic origin.

Aspiration pneumonia occurs after inspiration of foreign substances that enter respiratory tract from outside (e.g., from oral cavity). Aspirates may contain oral secretions, acidic gastric contents, vomiting masses,

foods, and multiple microorganisms. It starts as chemical and mechanical pneumonitis followed by progressive bacterial inflammation. Aspiration pneumonia often leads to lung abscess or pleurisy with empyema.

In most of cases this disease emerges as *hospital acquired pneumonia.* It is caused predominantly by gram-negative anaerobes, *S. aureus*, *Klebsiella pneumoniae* or *Pseudomonas aeruginosa.*

Ventilator-associated pneumonia or ***VAP*** is one more type of *hospital-acquired* (or ***nosocomial***) *pneumonia.* It may develop in patients undergoing mechanical ventilation within intensive care units.

Severe *hospital pathogens* demonstrating *striking resistance to antimicrobial therapy* are commonly isolated in case of VAP. Among them are methicillin resistant *Staphylococcus aureus* (or ***MRSA***), *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae*, and *Serratia marcescens*.

Laboratory diagnosis of nosocomial pneumonias includes specimen sampling followed by identification of isolated bacterial cultures and their antibiotic susceptibility testing.

Patient's sputum, airway samplings, endotracheal aspirates, bronchoalveolar lavage fluids, pleural fluid cultures are usually examined. Direct identification of bacterial strains in clinical specimens is performed by polymerase chain reaction (PCR).

The treatment of severe pneumonias caused by multi-resistant hospital strains requires *high-dosage combined antibiotic treatment* with broad-spectrum antibiotics.

Antipseudomonal cephalosporins (cefoperazone or ceftazidime), carbepenems, combined β-lactam/β-lactamase inhibitor-containing antibiotic piperacillin-tazobactam, fluoroquinolones (levofloxacin) or aminoglycosides (amikacin or tobramycin) are generally administered.

Gram-positive multi-drug resistant microflora, e.g. methicillin resistant *Staphylococcus aureus*, is treated with linezolid or vancomycin.

3.12. Odontogenic sepsis

Sepsis is *generalized* life-threatening *bacterial or fungal infection* followed by systemic body inflammation known as *systemic inflammatory response syndrome* or *SIRS.*

The term "*sepsis*" originates from *Greek*: "putrefaction" or "rotten flesh" indicating deep impairment of the whole body due to the severe infection.

Odontogenic sepsis is induced by infection residing in oral cavity. It becomes much more likely if odontogenic infection spreads through the fascial spaces to the head and neck.

Primary focus of infection may localize in mandibular (about 70%) or maxillary (for about 30%) zones. If not restricted, the infection can spread further to unaffected anatomical regions and tissues along the sites of least resistance.

When arisen, the septic state expands into systemic inflammatory disease via several consecutive *phases*. They reflect the levels of disease progression followed by growing incapacity of host defense to restore normal body functions.

The ***stages of systemic bacterial infection*** grow as follows: (1) *bacteremia* phase; (2) *sepsis*; (3) *severe sepsis*; (4) *septic shock.*

Bacteremia means the presence of *viable* bacteria in bloodstream. Nevertheless, bacterial dwelling in blood creates only necessary but not yet sufficient condition for sepsis development.

Bacteremia is generally divided into *transient*, *intermittent*, or *continuous* depending on duration of bacterial stay in blood.

Transient bacteremia lasts from a few minutes to few hours. It may occur under ordinary medical manipulations (i.e., dental extractions or percutaneous injections and catheterizations).

Intermittent bacteremia appears in case of presence of localized infectious focus such as abscess or osteomyelitis. It is characterized by periodical entry of bacteria into bloodstream with subsequent clearance.

Finally, *continuous* bacteremia occurs when the infection long-time resides directly within blood vessels or cardiac valves, i.e., in cases of infective endocarditis or vessel graft infections.

But the progression of conventional bacteremia into systemic disease or sepsis requires extra-conditions that must aggravate primary course of infection.

The bacteria should demonstrate high virulence with powerful adhesive and invasive capacity; on the other hand, host defense renders overexuberant but ineffective inflammatory response unable to clean the invaded pathogen.

Sepsis is defined as ***systemic inflammatory response syndrome*** (***SIRS***) that results from *documented* or *suspected* ***infection.***

It is the clinical syndrome originated from spreading infection that is followed by deep disorders in the inflammatory response as well as in local and systemic coagulation.

In 1992, the American College of Chest Physicians and the Society of

Critical Care Medicine approved the concept of sepsis as infectious systemic inflammatory response syndrome. On these grounds the definition of sepsis and severe sepsis was included into the list of 9th revision of International Classification of Diseases, Clinical Modification (ICD-9-CM).

Nevertheless, the term "SIRS" has more expanded meaning than sepsis itself.

SIRS denotes the syndrome of systemic body inflammation that should satisfy certain strict ***clinical criteria***:

- body temperature <36°C or >38°C;
- pulse rate >90 per minute
- respiratory rate >20 breaths per minute
- white blood cell count (WBCC) <4 or $>12*10^9$/L or normal WBC count, but with presence of more than 10% of immature cells.

According to these criteria, *SIRS* as systemic inflammation can arise from both *infectious* and *non-infectious* insults. For instance, non-infectious SIRS emerges in course of pancreatitis, burn disease, multiple trauma with massive tissue injury, or hemorrhagic shock.

On the other hand, sepsis concept presumes infectious SIRS. Thus, ***clinical definition for sepsis*** designates it as clinical condition manifested by *two or more of the SIRS criteria* (i.e., fever, tachycardia, tachypnea, or leukocytosis) associated with *proven or suspected infection.*

Pathogenesis of sepsis is based on abnormal hyperactivity of innate immune response against systemic infection. It is triggered by ***massive secretion of pro-inflammatory cytokines*** ("*cytokine storm*") from granulocytes, dendritic cells, macrophages and all other cells of innate response. This leads to endothelial damage, deep microcirculation disturbances and intensive procoagulant activity that ensure inner organs malfunction.

In case of ineffective treatment the disease progresses into *severe sepsis.*

Severe sepsis is defined as sepsis with *one or more inner organ failure.* It is followed by tissue hypoperfusion, oliguria, *disseminated intravascular coagulation* (or ***DIC***), thrombocytopenia, and hypotension (systolic blood pressure declines to < 90 mm Hg or reduces more than 40 mm Hg from baseline). In phase of severe sepsis patient's mortality rate strongly increases despite adequate antibiotic and infusion therapy as the

disease progression results in *septic shock* and *multiple-organ-dysfunction syndrome* (or *MODS*).

Septic shock is confirmed when established hypotension remains resistant to adequate fluid resuscitation. It is followed by perfusion abnormalities, lactic acidosis, oliguria, patient's mental disability and coma.

Overall, sepsis mortality rate remains high even in developed countries. In Europe it exceeds 40% in cases of severe sepsis. Nowadays the disease is called as "hidden public health disaster". For instance, there are about 750,000 patients with sepsis registered in United States annually resulting in more than 200,000 death cases; and the total cost of treatment of hospitalized patients is equivalent to $15 billion.

Laboratory diagnosis of sepsis including its odontogenic forms poses serious difficulties.

The diagnosis must be established as soon as possible (in first few hours) while massive early and targeted antibiotic therapy substantially favors the prognosis. The presence of initial infectious focus (e.g., necrotizing soft tissue infection) should be confirmed or excluded within 12 hours.

However, repetitive blood bacterial cultures become positive in less than 50% of cases. Primary culturing should be done before initiation of antimicrobial treatment. The most commonly isolated microbial species (e.g., staphylococci, viridance streptococci, gram-negative anaerobes, enterococci, *Pseudomonas aeruginosa*) are identified and tested for antibiotic resistance according to standard protocols.

Current progress in laboratory diagnosis of sepsis is related with methods of molecular genetic analysis. *Multiplex real-time PCR* tests and *fluorescence in situ hybridization* are used. Ideally they can detect individual microbial cells in 1 ml of blood.

Discovery of rapid reliable biomarkers of emergence of sepsis still remains the subject of intensive research. Laboratory monitoring of blood levels of biomarkers with relatively high specificity and sensitivity for sepsis (procalcitonin, C-reactive protein, lactate, IL-6 and some other cytokines) might be helpful in disease diagnosis and in control of treatment efficacy.

Treatment of odontogenic sepsis must be highly intensive and complex. It commences with complete surgical eradication of primary septic focus in orofacial or neck area.

Adequate and urgent antibiotic therapy that starts within 1 hour of sepsis diagnosis is the subject of ultimate clinical value. Initial empiric

antibiotic treatment is performed before the results of antibiotic susceptibility testing and it can be changed after collecting of microbial resistance data.

High doses of beta-lactams combined with β-lactamase inhibitors, carbapenems, aminoglycosides (amikacin or tobramycin), fluoroquinolones or vancomycin for gram-positive microflora can be used. In case of fungal sepsis amphotericin B is administered.

The treatment is followed by intensive maintenance of body vital functions – fluid resuscitation, raise of blood pressure, cardiovascular, respiratory and renal support.

Prophylaxis of odontogenic sepsis is essentially based on active elimination and treatment of primary infection focus located in oral cavity.

3.13. Bacteriological testing in dental practice

Bacteriological analysis in patients with odontogenic diseases is performed in several clinical conditions:

a) in cases of suppurative infections of orofacial and neck areas; the main goal of this study is the isolation of causative agent and its antibiotic susceptibility testing;

b) in clinical cases of "specific" infections, where oral cavity lesions arise in primary infectious diseases (e.g., diphtheria, syphilis, scarlet fever, tuberculosis and others); it fosters the establishment of correct etiological diagnosis of infection;

c) in cases of long-term oral lesions of unknown origin (unclear diagnosis).

Specimen taking for microbiology examination requires some common rules. Sampling should be done without any preliminary mouth treatment by antiseptics or other drugs and before tooth brush. Directly before taking oral cavity is rinsed with sterile warm saline. In case of oral ulcerative lesion its superficial covering film should be removed, and the specimen is delivered from lesion's bottom.

Without delay the material should be sent to the laboratory for culture examination. If proper nutrient media present, the inoculation can be done directly in dental cabinet.

Most common situations that demand bacteriological testing of odontogenic infections are related with cervical lymphadenitis, submandibular, retropharyngeal and other deep space infections, peritonsillar abscesses.

Specimen collection is made by aspiration or biopsy of inflammatory material from injured tissues and tissue spaces. Samples have to be placed into anaerobic transport container to make possible isolation of anaerobic bacteria, which are common in these conditions. Besides, facultatively anaerobic bacteria remain viable in anaerobic transport. All the specimens should be immediately delivered to the laboratory, where the isolation of microbial cultures and their antibiotic susceptibility testing are elaborated.

In odontogenic sepsis (as well as in other septic cases) the repeated blood sets taken from patient are examined. At least 2–4 blood culture sets are tested per septic episode.

For successful microbial recovery the most critical is the volume of blood that is collected by venipuncture. Not less than 20-30 ml of blood for every bottle with nutrient media should be taken. In adults 2-4 bottles for single blood culture set are used, at least one aerobic and one anaerobic. The amount of nutrient medium in every bottle has to be 200 ml or more to remit bactericidal activity of blood. Catheter-drawn blood cultures demonstrate a higher risk of external contamination.

In sepsis two and more blood culture sets must be taken sequentially in a short period of time before initiation of antibiotic treatment; after blood sampling empiric antibiotic therapy should be started immediately.

For *rapid sepsis diagnosis* nucleic acid amplification tests (*NAATs*) are used. Patients' plasma or serum is examined by multiplex real-time PCR or molecular hybridization tests.

Microbiological testing of saliva and gingival crevicular fluid ameliorates the diagnosis of periodontal diseases and helps to assess risks in patients with caries.

Saliva testing can be done both with resting (unstimulated) or stimulated salivary specimen.

Resting saliva is secreted predominantly by submandibular glands. It is collected by allowing a patient to expectorate saliva into a collection cup.

Stimulated saliva is derived mainly from the parotid gland; it can be taken by masticatory stimulus (e.g., a piece of wax). To get stimulated salivary specimen, the patient chews a piece of wax for a 5 min expectorating saliva into a small receptacle at regular time periods.

Salivary samples are used for rapid chairside caries risk assessment tests. Risk assessment tests estimate total amount of the most cariogenic bacterial species, namely *S. mutans* and lactobacilli, by reaction of salivary aliquot with highly specific monoclonal antibodies against these pathogens.

Gingival crevicular fluid (GCF) is tested for laboratory diagnosis of periodontal diseases. GCF samples are obtained from periodontal lesions (e.g., gingival pockets) of the tested teeth. The teeth should be cleaned to remove any supragingival plaque, then isolated with cotton roll to prevent saliva contamination and dried with air. Standard endodontic paper points (e.g., of size 30) or collection paper stripes are inserted into the gingival crevice. Standardization is achieved by equal time of sampling.

Next step the obtained specimen is transferred to appropriate nutrient media for growth. Anaerobic culturing is preferable in this condition as the most active periodontal pathogens (i.e., *Prevotella intermedia, Tannerella forsythia* or *Porphyromonas gingivalis*) pertain to obligate anaerobic bacteria.

Swabs of dental plaques are used to evaluate the grades of common oral cleanliness. For example, the quantity of microbial ATP can be determined in this sample that correlates with total amount of bacteria present in dental biofilm.

IV. INFECTIOUS DISEASES WITH SPECIFIC LESIONS IN ORAL CAVITY

4.1. Tuberculosis

Tuberculosis is one of the most severe threats for human health at the beginning of XXI century. Annual total number of disease cases is about 9 million. It is generally assumed that near one quarter of affected persons dies from tuberculosis and its consequences, and most of patients are young adults. Moreover, mortality rate in untreated or untreatable disease exceeds 50%. Thus, tuberculosis remains the leading infectious cause of human death resulting in more than 1.3 million lethal cases every year.

Moreover, the current increase of new tuberculosis cases is about 2% per year, but the rise of *multi-drug resistant* (*MDR*) *tuberculosis* and *extensively drug-resistant tuberculosis* (*XDR*) is much more rapid. Now about 3% of tuberculosis cases worldwide are produced by MDR mycobacteria, and in certain world regions (African countries, several Chinese and Russia provinces, Baltic states, etc.) their incidence exceeds 10%. Therefore, the global spread of MDR tuberculosis is a problem of great medical and social importance.

The main sources of infection are persons with active tuberculosis. Sick animals (cattle) can also spread the disease.

Tuberculosis of oral cavity is a seldom clinical situation among common lesions of oral cavity. Nevertheless, every case of oral ulceration requires stringent differential diagnosis for tuberculosis.

Tuberculous oral injuries can be *primary* or *secondary to pulmonary disease*; the latter are much more frequent. Oral lesion in tuberculosis can resemble stellated ulcer. In many cases it affects the dorsum of tongue. The ulceration is usually painful and demonstrates undermined ragged edges and granulated base. Less often the lesions might be found on gingiva, lips, buccal mucosa, palate, or floor of mouth.

The differential diagnosis of oral tuberculosis is conducted with aphthous stomatitis, syphilitic lesions and oral malignancies such as squamous cell carcinoma or lymphoma. In this condition biopsy sampling is mandatory.

Laboratory diagnosis of disease is based on microscopy of specimen, taken from oral lesion, and isolation of *M. tuberculosis* on selective media. Ziehl-Neelsen acid-fast bacilli stain or fluorescent microscopy with auramine stain is used.

In most of cases oral tuberculosis is secondary to pulmonary disease, that's why sputum culture must be examined.

Specific treatment of tuberculosis grounds on long-term regimen of combined antimicrobial chemotherapy. It lasts up to 6 months. The list of first-line drugs comprises isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin. If necessary, oral mycobacterial lesions are subjected to surgical treatment.

4.2. Actinomycosis

The patients with ***actinomycosis*** of orofacial area comprise up to 80% of all infection cases. The main causative agents of the disease are *Actinomyces israelii* and *A. gerencseriae*. They are commonly associated with *Aggregatibacter actinomycetemcomitans*, *Aggregatibacter aphrophilus* and propionibacteria.

In relatively large amounts actinomycetes reside in dental plaques of clinically healthy individuals. Also they are present in soil, contaminate herbs and grains. In this vein actinomycosis contraction occurs from *endogenous* or *exogenous* microbial infection.

The disease transmission is possible by airborne, contact or rarely by alimentary route. After initial contact with skin or oral mucosa the bacteria activate their lymphogenous or hematogenous spread into deep tissues. They can reach adipose tissue, muscles and fascia resulting in formation of long-term granulomatous and poorly healing abscesses. In case of progression the abscesses enlarge and may open spontaneously with purulent discharge.

Primary actinomycosis is readily complicated by secondary infection predominantly of anaerobic genesis.

Microbiological examination starts from microscopy of purulent discharges taken from inflammatory abscess lesions. Round-shaped branched microcolonies of actinomycetes are indicated. Their inner structure resembles mycelium – fungus-like branched network of hyphae.

Successful treatment of actinomycosis requires high-dose administration of antimicrobial drugs for which the bacteria retain sensitivity: beta-lactams, macrolides, or doxycycline.

4.3. Diphtheria

Diphtheria is the severe infectious disorder caused by toxigenic *Corynebacteria diphtheriae.*

Bacterial exotoxin plays the principal role in the pathogenesis of disease, blocking intracellular protein synthesis in tissues and organs.

Diphtheria is manifested by characteristic *fibrinous inflammation* with growing vascular penneability. It results in formation of tight "pseudomembranes" covering the tonsils, pharynx, or larynx. Proteinaceous pseudomembranes contain fibrin that is firmly attached to innermost tissues.

Diphtheria is anthroponotic disease. Patients with diphtheria and carriers are the main sources of infection. The disease is communicated by airborne route.

Exotoxin traverses the mucous membranes and causes the destruction of epithelium with inflammatory response and microcirculatory and coagulation disorders. The expanding necrotic pseudomembranes impede normal airflow. Any attempt to remove the pseudomembrane results in bleeding. Pseudomembrane respiratory obstruction (or *diphtheritic croup*) can cause patient suffocation. The regional neck lymph nodes enlarge and neck swelling progresses resulting in total neck edema ("bull neck").

Toxin absorption leads to distant toxic action with tissue damage, parenchymatous degeneration, fatty infiltration and necrosis in myocardium, liver, kidneys, and adrenals, sometimes accompanied by hemorrhage. The toxin also produces nerve damage often followed by paralysis of the soft palate, eye muscles, or limbs.

In dental practice the most common is the local form of diphtheria where pseudomembranes cover patient's tonsils.

Toxic and hypertoxic clinical forms are characterized with burst progression of the disease resulting in toxic shock that may cause patient death in two-three days.

Laboratory diagnosis of diphtheria depends on rapid determination of diphtherial exotoxin and isolation of toxigenic bacteria.

The presence of exotoxin in clinical specimen (primarily, in tonsillar pseudomembranes) is detected by enzyme-linked immunosorbent assay (ELISA test) or by immunoprecipitation. The toxigenicity of *C. diphtheriae* culture can be shown also by incorporation of bacteria into cell culture monolayers. Toxin diffuses into cells monolayer and causes cell destruction. Finally, PCR is used as the most sensitive, rapid and specific method for determination of gene encoding diphtheria toxin.

The treatment of diphtheria basically rests on the early administration of specific antitoxic antibodies that block toxin action.

Specific prophylaxis is afforded by active immunization. Usually combined DPT (or diphtheria-pertussis-tetanus) vaccine or combined tetanus-diphtheria toxoids are used. All the children must gain the repetitive course of diphtheria toxoid immunizations followed by several boosters in every 10 years.

4.4. Scarlet fever

Scarlet fever is acute *streptococcal infection* caused by group A *Streptococcus pyogenes* that produce *pyrogenic exotoxins A, B and C*. The symptoms of the disease ensue from systemic toxin action. Toxins display superantigenic activity with massive production of proinflammatory cytokines. This results in fever, generalized rash, and skin desquamation. The disease profoundly impairs cardiovascular system especially microcirculation.

Scarlet fever transmission occurs by air-droplet route. The illness affects predominantly children. It begins from sharp raise of temperature, vomiting and throat pain. In 1-2 days characteristic skin rash appears and moves down from face to trunk and limbs. The patient demonstrates bright red cheeks and chin with a typical pale area around the mouth. The rash stays for several days and then gradually fades with skin desquamation (or peeling).

Almost all cases of scarlet fever are followed with specific oral lesions such as "strawberry" tongue with elevated deep-red lingual papillae, Forchheimer spots (fleeting small red spots covering the soft palate) and inflamed uvula.

Clinical diagnosis of the disease is supported by isolation of group A streptococci from patient's throat swab. Microbial culturing is performed on number of special media for streptococci (blood or serum agar).

Scarlet fever is efficiently treated with antibiotics. Beta-lactams are the most commonly used antimicrobials here because of retained sensitivity of *S. pyogenes* to this group of drugs.

4.5. Anthrax

Anthrax is the severe zoonotic disease caused by toxigenic *B. anthracis*. Herbivores can become infected with anthrax by grazing in

pastures that are contaminated with spores. It results in bacterial propagation within animal intestine with subsequent microbial discharge. This leads to soil contamination with vegetative microbial cells. They sporulate and persist in the soil for 50 years and even more. Animal carcasses are highly infectious. Biting flies can become vectors for the spread of anthrax. Contact with animals (butchering, skinning, or exposure to hides or wool), and consumption of contaminated meat are risk factors for infection in humans.

Depending on the site of the infection, anthrax cases have very different clinical manifestations – cutaneous anthrax, inhalation anthrax (or wool-sorter's disease) and gastrointestinal disease.

Cutaneous anthrax is the most frequent form of disease (95% of total cases); and the skin of face and neck becomes the portal of entry for infection in approximately 40% of patients. Therefore, it requires thorough differential diagnosis with other neck and facial lesions.

In *cutaneous anthrax*, spores are introduced into the skin. Germination occurs within hours, and vegetative cells produce anthrax toxin. The disease usually develops within 1-7 days after entry.

A red macule emerges at the site of inoculation. The lesion subsequently comes into a papular-vesicular stage that is followed by ulceration with a blackened necrotic *eschar* or *anthrax carbuncle* (malignant pustule) surrounded by brawny edema. This lesion is painless. A regional lymphadenitis is commonly seen in these patients. Eventually eschar dries, loosens, and separates; spontaneous healing occurs in 80 to 90% of untreated cases. However, in severe cases bacterial dissemination leads to septicemia with high mortality rate.

Anthrax diagnosis relies on clinical findings and disease epidemiology with subsequent microbiological confirmation. It includes microscopy of samples taken from primary skin lesions and bacterial isolation.

The patients are hospitalized and treated with antibiotics (fluoroquinolones or beta-lactams) and specific immunoglobulin.

4.6. Syphilis

Primary syphilitic lesions in oral cavity may inflict buccal and gingival mucosa, lips or perioral skin. They emerge in affected individuals after oral sex. The rates of other routes for disease transmission (via personal things such as tooth brushes, or by direct contact, or after medical manipulations) are negligibly low.

After sexual intercourse the causative agent of syphilis *T. pallidum* invades the skin or mucosals through their minimal lesions. Infectious dose for it is extremely low: as little as 1-5 microbial cells can trigger the illness.

Incubation period depends on inoculated dose. A large inoculum, e.g., about 10^7 bacterial cells, results in disease appearance in 5-7 days.

After approximately 1 month of incubation a *hard chancre*, essential tissue lesion of *primary syphilis*, appears. It is followed by regional lymphadenopathy.

Chancre evolves at the primary site of microbial entry. Its orofacial localization often occurs on lip vermilions and oral mucosa. Hard chancre is a painless ulcer about 0.5-3 cm with sharp margins, clean base, induration, and sometimes with purulent discharge.

In most cases chancre heals spontaneously within about 6 weeks. Nevertheless, in several weeks the disease comes into stage of "*secondary syphilis*", which results from lymphogenous and hematogenous microbial dissemination.

Secondary syphilis is the systemic inflammatory process characterized by skin rash, headaches, fever, malaise, lymphadenopathy, mucosal lesions, and CNS disorders. It lasts from 2-3 months to more than 1 year. Cutaneous or mucosal syphilitic eruptions or *syphilides* harbor great amount of spirochetes, being highly infectious.

In oral cavity secondary mucosal lesions appear as the erythematous and maculo-papular syphilides sometimes known as mucous patches. They render oval-shaped grayish-white elements on mucosal surface followed by periostitis. Striking variability of secondary lesions makes the diagnosis seriously difficult.

If not treated, after latent period of various length (about 1 year or even more) the disease progresses into *tertiary syphilis*.

Tertiary syphilis affects various body's organs and tissues, especially cardiovascular system and CNS. Specific slow-growing indurative injuries (or *gummas*) emerge in tissues and parenchimatous organs resulting in necrosis with subsequent connective tissue proliferation.

These lesions rarely appear in mouth. If arisen, they form growing nodules (tubercular syphilides) and gummas. When progressed, the lesions undergo deep necrosis with degradation of underlying soft tissues and bones, for instance, resulting in perforation of the soft palate.

Congenital syphilis in infants issues from vertical disease transmission in untreated women with a rate of 70 to 100% for primary syphilis. The disease seriously impact on pregnancy outcome, thus it is often followed

by spontaneous abortion, or perinatal death. The infected infants may be asymptomatic or show the numerous manifestations of early and late congenital disorder with multiple dental abnormalities. Herein they render screwdriver-shaped incisors with notched incisal edges (Hutchinson's incisors). First molar cusps remain underdeveloped. Mulberry deformation of molars is observed.

Overall, *Hutchinson's triad* of abnormalities in congenital syphilis comprises lymphadenopathy and hepatomegaly accompanied with skeleton and teeth lesions.

Laboratory diagnosis of syphilis rests on microscopical examination of specimens taken from primary or secondary syphilitic lesions and/or serological tests for specific antibodies.

Serologic testing is the mainstay of laboratory diagnosis for latent, secondary, and tertiary syphilis. It confirms the presence of anti-treponemal antibodies in patient sera by means of highly specific serological reactions, e.g., *T. pallidum* immobilization test and ELISA test.

The treatment of the disease is based on high sensitivity of *T. pallidum* to beta-lactam antibiotics. Penicillin G and its derivatives remain to be the drugs of choice for syphilis treatment.

4.7. Gonococcal infection

Gonococcal stomatitis can arise in newborns infected from sick mothers in the course of delivery. In adults oral gonococcal infection usually follows urogenital infection in persons practicizing orogenital contacts.

Mucosal tissue, tonsils, pharynx and upper larynx are involved into inflammatory process. Inflammatory manifestations result in erythematous edema, suppurative erosions and ulcerations of oral mucosa.

Laboratory diagnosis of gonococcal infections is carried out by microscopy of the materials obtained from urogenital excretions and lesion discharges.

Antimicrobial treatment is performed by antibiotics with proven efficacy against gonococci because of high levels of resistance essential for these bacteria. Combinations of azithromycin with gentamycin or gemifloxacin are preferentially used. Local treatment of specific oropharyngeal lesions with antiseptics can be applied.

4.8. Oral candidiasis

Oral candidiasis (or candidal stomatitis, oropharyngeal candidiasis and moniliasis) is the specific oral mycosis caused by yeast-like fungi of *Candida* genus.

More than 50% of disease cases is related with commonly spread fungi ***Candida albicans***; other causative species for candidiasis in descending rate are *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, or *C. krusei*.

C. albicans pertain to the normal representatives of oral microflora, where they can be found in modest amounts. However, in some specified conditions these fungal members exert serious *opportunistic infections* rendering local or severe systemic disease..

C. albicans are *dimorphic fungi*. In mouth they are often present as *yeast forms* or blastospores with questionable virulence whereas mould-like fungal *hyphal forms* are capable of invading host tissues. Transition between these two phases largely depends on changes of environmental conditions.

In addition candidae create *pseudohyphae* – long filaments composed of oval fungal cells that are closely attached on their poles. It occurs due to incomplete separation of cells after division.

A and B serotypes are identified by serological testing of *C. albicans*. Representatives of A group are characteristic for normal oral microbiota; by contrast, the members of B group arise actively in immunocompromised hosts.

Overall, all the details of transformation of oral saprophytic candida into aggressive fungal pathogens are not yet clear completely.

It is considered that candidal strains with enhanced capacity to adhesion and colonization are generally more pathogenic. In particular, the strains with high expression of certain adhesins (e.g., hyphal wall protein 1 or extracellular mannoprotein) are referred to as more active pathogens. Similarly, pathogenic fungi produce large amounts of hydrolytic enzymes such as proteases, phospholipases and hyaluronidase.

Oral candidiasis is regarded as the *most frequent opportunistic infection of oral cavity* that affects humans. Likewise, it is the most typical form of candidal infection and the most common form of oral mycosis at all.

Candidiasis affects newborns and infants, but most of all – inmunocompromised persons, namely patients with HIV infection and acquired immunodeficiency syndrome (AIDS); patients with cancer under cytostatic chemotherapy; patients, treated with antibiotics of broad

spectrum of action with deep oral or intestinal dysbiosis.

Primary carriage of *C. albicans* predisposes to its further opportunistic infection.

By far, elevated candidal carriage is typical for persons, wearing dentures; patients, aggressively treated with broad spectrum antibiotics or immunosuppressive drugs; HIV patients; diabetes and cancer patients; patients with "dry mouth" (or xerostomia); individuals with abundant consumption of sugar-containing nutrients, heavy smokers and some others.

Thus candidiasis is often named as "a disease of the diseased".

Candidal infection is divided into acute and chronic, primary and secondary.

Primary oral candidiasis originates from resident fungi and affects oral cavity and perioral area. By contrast, *secondary* disease emerges after spread of disseminated candidal infection that occupies the mouth and other body compartments.

Nevertheless, dissemination of oral candidiasis to other body sites (or invasive infection) is generally seldom situation. It happens predominantly in immunocompromised patients.

There are 3 main forms of oral candidiasis:

(1) pseudomembranous;

(2) erythematous;

(3) hyperplastic.

Pseudomembranous candidiasis, or *thrush*, demonstrates white pseudomembranous spots upon oral mucosa that contain epithelial and fungal cells mixed with fibrin and cellular decay. They resemble "cottage cheese" or "curdled milk". The covering film is easily removed showing red mucosal bottom. These lesions usually affect tongue, palate and buccal epithelium.

Acute pseudomembranous oral candidiasis is registered in 5% of newborns and early infants. They become infected in delivery by contact with maternal vaginal canal harboring candidae.

Chronic pseudomembranous candidiasis arises mainly in immunocompromised persons, e.g., HIV patients.

Erythematous form of the disease exposes typical smooth and reddened lesions, which are located mainly upon the tongue dorsum or palate. The lesions are usually painful. Substantial loss of lingual papillae (or depapillation) is common here.

In most of cases this fungal infection is the result of long-term antibiotic treatment or corticosteroid therapy. That's why it is often named

as "antibiotic induced stomatitis" or "antibiotic sore mouth".

Erythematous illness covers up to 60% of total oral candidiasis if taken with related clinical forms such as angular stomatitis and denture-related stomatitis. The latter is associated with chronic erythematous candidiasis.

Hyperplastic candidiasis is a seldom form of disease with the incidence of about 5%. It is named as nodular or plaque-like candidiasis. This variant produces white firm nodular lesions predominantly found upon both sides of buccal mucosa.

Candida-associated lesions are the oral injuries caused by candida fungi in association with other pathogenic microorganisms. Two basic forms are known – angular cheilitis and denture related stomatitis.

Angular cheilitis is the infection-based inflammation at mouth angles. About 20% of disease is caused solely by candidal species, whereas 60% originate from association of *C. albicans* and *S. aureus*. This pathology renders angular soreness and erythematous inflammation that may result in angular fissuring. The syndrome affects elderly adults and often accompanies denture related stomatitis

Denture related stomatitis is a low or moderate inflammatory complication that affects edentulous elderly adults wearing oral appliances (*dentures*).

It is generally ascertained that more than 50-60% of denture-wearing individuals exhibit denture related stomatitis.

Dominating causative agents in this pathology are candida fungi (above 90% of total number of cases). Thus the disease is commonly termed as "*Candida-associated denture induced stomatitis*" or CADIS.

Mucosal surface under dentures demonstrates highest grades of fungal colonization in comparison with normal unaffected mucosa. It creates acidic, moist and relatively anaerobic surrounding that promotes further candidal growth. In addition, poor adjustment of oral prostheses causes micro-injuries of gingival mucosa.

Candidae easily attach to the surface of damaged tissues and polymeric surface of dentures with their multiple pits and fissures. Microbial adherence and colonization stimulates local inflammation. This leads to continuous irritation of oral mucosa resulting in erythematous lesions.

Chronic denture related stomatitis creates the stable reservoir for candidal infection that in worsen conditions may expand to other areas of oral cavity.

Diagnosis of oral candidiasis grounds on clinical findings but requires

laboratory confirmation of fungal infection.

Sampling is made from lesion sites by oral smears, swabs or rinsing.

Microbial smears are examined by Gram stain demonstrating gram-positive candidal cells with pseudohyphae.

Fungal culture is made on *Sabouraud medium*.

Oral rinse examinations help to discriminate between "normal" candidal carriage and oral candidiasis. About 7,000-7,500 colony-forming units of candidae per 1 ml of oral rinse can be found in disease condition.

Treatment of oral candidiasis presumes administration of topical anti-fungal drugs, such as miconazole, nystatin, levorin, or amphotericin B.

Severely immunocompromised patients, e.g., with candidiasis in AIDS, need systemic anti-fungal therapy with oral or intravenous drugs (amphotericin B, azoles or others).

Proper oral hygiene strongly reduces candidal propagation in oral cavity. It presumes adequate toothbrushing, smoking cessation, oral rinsing after inhalation steroid use, and regular denture disinfection with denture cleaner preparations (e.g., chlorhexidine or sodium hypochlorite).

4.9. Viral infections, affecting oral cavity

4.9.1. General traits of viral infections targeting oropharyngeal area

Viral lesions are considered among the most common in current dental practice.

Numerous viral infections cause specific alterations within oral cavity. It can be illustrated by exuberance of clinical cases. For instance, in severe influenza course hyperemia and cyanosis of oral mucosa are typical. Diffuse bright hyperemia affects oropharynx (including tonsils and arches) and spreads to soft palate. Minor granularities of palate mucosa, sometimes of uvula and arches are visible, accompanied by petechial microhemorrhages.

Infectious mononucleosis is followed by tonsillitis with multiple *petechial rashes* that covers oral mucosa. Likewise, oropharyngeal lesions are common in parainfluenza infection, in rubella, rhinoviral and adenoviral diseases. Manifestations in viral hemorrhagic fevers comprise severe hemorrhagic rash, cheilitis, angular stomatitis, catarrhal gingivitis.

Herpes simplex virus, vesiculovirus and herpes zoster virus, coxsackie A viruses, virus of vesicular stomatitis are able to cause acute, chronic or latent infections with similar alterations in oral cavity. Primary lesion here

is *vesicle* that is gradually changed into ulcerative erosion or *aphtha*.

4.9.2. Herpetic oral lesions

4.9.2.1. Herpes simplex infection

Viral infection, caused by 1st type of herpes simplex virus (***HSV-1***) is the most common viral disease in humans. By sensitive laboratory tests herpes simplex persistency is detected among 90% of adult population.

Infants in the age range from 6 month to 3 years are grossly susceptible to this infection. Large outbreaks of acute herpetic stomatitis emerge in child care settings.

The main clinical presentations of infection are *acute (primary) herpetic gingivostomatitis* and chronic *herpes labialis* (or *cold sores*) with its recurrent exacerbations.

Acute herpetic gingivostomatitis occurs as initial (primary) herpetic infection. It is caused by HSV-1 and in rare cases by HSV-2 that is common for genital infection.

Incubation period lasts for 4-5 days. It is followed by fever and appearance of characteristic lesions – pin-head vesicles, localized on lips, tongue and gingival or buccal mucosa. Soon the vesicles become eroded forming painful ulcerations with yellow-grey surface. Virus-induced inflammation involves regional lymph nodes resulting in submandibular lymphadenitis.

Usually isolated viral lesions heal spontaneously in several days without scarring. Clinical recovery occurs in 1-3 weeks.

Nonetheless, in course of primary infection the virus invades local nerve endings and moves by retrograde axonal flow to dorsal root ganglia, where the *latency* is established. Oropharyngeal HSV-1 infections result in *viral persistence* within the *trigeminal ganglia*. The virus resides here in a non-replicating state. Within infected ganglia herpetic infection stays life-long.

*Chronic **herpes labialis*** (or *cold sores*) results from repetitive exacerbations of latent HSV-1 infection. In most of cases various exogenous stimuli (fever, UV-irradiation, physical or emotional stress, axonal injury, etc.) activate viral replication. The virus moves along axons back to the peripheral site; and replication proceeds at the skin or mucous membranes. The vesicles commonly affect perioral area, lip vermilions and their borders, sometimes – soft and hard palate, tongue or buccal epithelium. Many recurrences are asymptomatic.

Clinical diagnosis of herpetic infection is confirmed with *laboratory*

testing of specimens taken from herpetic lesions. Immune fluorescence analysis and PCR are the standard reactions in this condition. Serological testing states the elevated levels of specific anti-viral antibodies of IgM class.

In mild or moderate cases acute herpetic gingivostomatitis doesn't require *treatment*. Severe or complicated manifestations can be treated with topical applications of acyclovir on the background of adequate oral hygiene.

However, HSV remains latent in sensory ganglia, and the rate of relapse is similar in drug-treated and untreated individuals.

The treatment of recurrent herpes labialis tends to restrain active disease exacerbations. In addition to antiviral therapy antiseptics are used to prevent secondary bacterial infections. Several vaccine candidates are under experimental or clinical testing now.

4.9.2.2. Varicella-zoster herpesvirus infection

Varicella-zoster or *type 3 herpesvirus* causes two clinical forms of the disease. One is ***chickenpox*** (or ***varicella***) that afflicts primarily non-infected individuals, predominantly children or young adults.

Second disease or ***shingles*** (also known as ***zoster*** infection) arises from occasional re-activation of latent varicella-zoster infection usually in many years after primary infection episode. More than half of cases are registered in people above 50 years or immunocompromised persons. The disease is characterized by severely painful vesicular rash emerging in the skin areas innervated by certain spinal or cranial nerve.

Primary varicella infection is transmitted via air droplet route. The portal of entry is the mucosa of upper respiratory tract or the conjunctiva. The virus circulates in blood, undergoes multiple cycles of replication with eventual localization in the skin and mucosal tissues. Focal cutaneous and mucosal lesions are observed.

The incubation period of *varicella* is about 10-21 days. Malaise and fever are the earliest symptoms, soon followed by vesicular rash on the skin of trunk, face, or limbs, as well as on buccal, gingival or pharyngeal mucosa.

The patient remains contagious until crusting of all the lesions, usually in 5-6 days. Varicella complications are rare.

Previous infection with varicella is believed to confer lifelong immunity to varicella.

The skin lesions of ***shingles*** (or ***zoster***) are highly similar to those of varicella.

It is not clear what triggers reactivation of latent varicella-zoster virus infection in ganglia. It is supposed that insufficient immunity allows viral replication to occur in a ganglion. Virus migrates down the nerve to the skin and induces vesicle formation.

The disease usually starts with severe pain in the area of skin or mucosa, which is supplied by the group of sensory nerves and ganglia. After onset a chain of vesicles appears over the skin or mucosal tissue along the affected nerve. These lesions may occur in various sites including oral cavity. The blisters fill with lymph, than open and crust over.

The most common complication of zoster in the elderly is *postherpetic neuralgia*. The pain may continue for weeks to months.

Second exacerbation of shingles in the same person is rare, being most likely in patients with immunodeficiencies. Therefore, cell-mediated immunity is regarded as the primary defense barrier against varicella-zoster virus.

Diagnosis of varicella-zoster infection grounds on clinical findings and laboratory testing for virus. In latter case viral DNA is detected in specimens from vesicle fluid by PCR.

Serological testing reveals the raise of anti-viral IgM antibodies in chickenpox or IgG class antibodies in case of shingles.

Varicella is usually a mild disease and requires no specific *treatment*, except complicated forms of disease in immunocompromised patients.

A number of antiviral drugs is effective against varicella, including acyclovir, vidarabine, and human leukocyte interferon.

In shingles antiviral therapy remits the severity and shortens the duration of infection. Acyclovir and more recent drugs valaciclovir and famciclovir are used in these situations.

A live attenuated varicella vaccine is administered for *prophylaxis* of chickenpox. Live attenuated vaccine against zoster infection is recommended for immunization of elder people over 60.

4.9.3. Herpangina

Herpangina (also known as *mouth blisters* or vesicular pharyngitis) is typically caused by ***Coxsackie group A*** viruses. In few cases it can be triggered by Coxsackie group B infection and echoviral infection. All these viruses are the members of *Enterovirus* genus and *Picornaviridae* family.

Coxsackie herpangina is a severe febrile pharyngitis. It affects predominantly babies and young children.

The disease contracts by air droplet and fecal-to-oral routes.

There is an abrupt onset of fever and sore throat with discrete vesicles on the posterior half of the palate, pharynx, tonsils, or tongue. The vesicles progress into ulcerations that heal spontaneously in 7-8 days.

The disorder is self-limited and resolves in 1-2 weeks.

Diagnosis rests on clinical findings. The treatment of herpangina is symptomatic; specific antiviral therapy is absent.

4.9.4. Oral lesions in measles

Measles is an example of extremely contagious acute respiratory viral infection. It is caused by specific *morbillivirus* of a single serotype. Measles is a human illness that usually affects children.

Virus replicates in respiratory tract epithelium and moves to regional lymphatic nodes (primary viremia). After second propagation in the lymphoid tissue it spreads throughout the body penetrating endothelium of vessels and epithelial cells in skin, conjunctiva, respiratory tract, and oral cavity.

Characteristic lesions of oral cavity in measles are known as ***Koplik's spots***. They appear upon the buccal mucosa two to three days earlier than the measles rash. The spots are the sites of intensive viral replication with formation of giant cells.

Koplik's spots can be discerned as firm white lesions on the buccal epithelium opposite to the lower 1st and 2nd molars. They are highly specific for the disease, sometimes described as "grains of salt on a wet background".

As Koplik's spots emerge at the beginning of measles, their discovery makes possible timely isolation of contact individuals thus preventing further disease spread.

4.9.5. Oral manifestations of HIV infection

Severe immune suppression that follows progressive ***HIV infection*** results in development of ***acquired immune deficiency syndrome***, or ***AIDS***. Similar with other body compartments, HIV dampens immune response within oral cavity. This creates conditions for local oral manifestations of opportunistic infections and tumors.

Before the clinical implementation of highly active antiretroviral therapy (or HAART) in 1996, oral lesions were registered in 50% of individuals infected with HIV and in about 80% of persons with AIDS.

Since that time the course of HIV infection has become more benign but the oral lesions remain to be common in this patients.

The clinical value of diagnosing and treatment of oral lesions in persons with HIV was summarized by M.M. Coogan in 2005. It comes from a number of findings, which are characteristic for oral manifestations in HIV infection.

1. Can help to diagnose the presence of HIV infection in otherwise healthy individuals.
2. Develop early in an infection.
3. Help to determine the progression of HIV infection to AIDS.
4. May serve for entry and end-points in vaccine trials.
5. Can be used in staging and classification of HIV diseases as determinants of opportunistic infection and anti-HIV therapy.

In 1992 the EC-Clearinghouse on oral problems related to HIV infection and WHO Collaborating Centre on Oral manifestations of the immunodeficiency virus work out the classification of oral manifestations of HIV infection. Later, in 2002, an international workshop confirmed the validity of this classification and recommended it for practical use.

Classification data summarizing oral lesions associated with HIV/AIDS are present in Table 3.

The most common oral lesions in HIV/AIDS are the results of ***candidal infection***. They occur at least in 40-60% of AIDS patients. The main causative agent is *Candida albicans*; it often associates with *C. tropicalis*, *C. parapsilosis* and others. *Erythematous* and *hyperplastic* candidiasis, *chronic pseudomembranous* disease, and *angular stomatitis* are typical in AIDS.

Viral infections in HIV/AIDS rapidly progress in condition of severe cellular immune deficiency. The viruses from ***Herpesviridae*** family play pivotal role in genesis of viral complications in AIDS.

Among them are herpes simplex and herpes zoster viruses, herpesvirus type 4 or Epstein-Barr virus, cytomegalovirus (herpesvirus type 5), and herpesvirus type 8 or Kaposi's sarcoma-associated herpesvirus.

Active reproduction of *Epstein-Barr virus* in epithelial cells of tongue exerts its specific lesion, known as ***hairy leukoplakia***. It follows about 50% of HIV infection cases.

Hairy leukoplakia represents white wrinkled lesions from lateral sides of tongue that are not painful. In most of cases it doesn't need specific treatment. Re-emergence of hairy leukoplakia in the same patient may indicate the failure of HIV specific therapy.

Table 3.
Oral lesions, associated with HIV/AIDS

Group 1. *Lesions strongly associated with HIV infection*	*Group 2.* *Lesions less commonly associated with HIV infection*	*Group 3.* *Lesions seen in HIV infection*
Candidiasis Hairy leukoplakia Kaposi's sarcoma Non-Hodgkin's lymphoma ***Periodontal diseases:*** linear gingival erythema, necrotizing ulcerative gingivitis, necrotizing ulcerative periodontitis	***Bacterial infections:*** Mycobacterium avium-intracellularae Mycobacterium tuberculosis Necrotizing (ulcerative) stomatitis Salivary gland disease Dry mouth due to decreased salivary flow Unilateral/bilateral swelling of salivary glands Non-specific ulcerations ***Viral infections:*** Herpes simplex virus Human papillomavirus Condyloma acuminatum Varicella-zoster virus	***Bacterial infections:*** Actinomyces israelii Escherichia coli Klebsiella pneumonia Cat-scratch disease Bacillary angiomatosis **Fungal infection other than candidiasis:** Cryptococcus neoformans Geotrichum candidum Histoplasma capsulatum Mucoraceae fungi Aspergilus flavus ***Neurological disturbances:*** Facial palsy Trigeminal neuralgia

Herpesvirus type 8 causes angioproliferative tumor ***Kaposi's sarcoma.*** It affects one third of patients with AIDS. About 40% of them demonstrate oral lesions. This vascular tumor renders multiple red-violet macules, papules or nodules mainly upon hard palate.

Repetitive herpes zoster infections are also common in AIDS patients.

Deep immune suppression in patients with AIDS-related complex and AIDS activates numerous opportunistic pathogens resulting in severe mixed bacterial, fungal and viral infections.

Among the most common are tenacious periodontal disorders of polymicrobial origin.

Usually periodontal damage starts from ***linear gingival erythema*** or *HIV-associated gingivitis*. It may progress further into ***necrotizing ulcerative gingivitis*** and ***necrotizing ulcerative periodontitis***.

Linear gingival erythema looks like narrow red band surrounding the marginal gum. If not controlled, it stimulates progression of *necrotizing ulcerative gingivitis* with deep damage of papillary gingival area and *necrotizing ulcerative periodontitis* with dental attachment loss and destruction of alveolar bone.

Periodontal pathology in AIDS arises from the joint action of typical oral pathogens, such as gram-negative obligate anaerobes *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Treponema denticola*, *Fusobacterium nucleatum*, *Actinomyces naeslundii*, and others. *Aggregatibacter actinomycetemcomitans*, candidae fungi, and viruses are also the active participants of AIDS-associated periodontal diseases.

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Генералов Игорь Иванович

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для студентов стоматологических факультетов высших медицинских учебных заведений

Пособие

Редактор И.И. Генералов
Технический редактор И.А. Борисов
Компьютерная верстка А.К. Гайлит
Корректор Н.В. Железняк

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