31. PROTOZOA FOUND IN THE ORAL CAVITY

The following are examples of protozoa that can be isolated from the oral cavity: *Entamoeba gingivalis, Trichomonas tenax, and Giardia lamblia.*

31.1. Entamoeba gingivalis

Classification. *Entamoeba gingivalis* is classified as a commensal microorganism from the genus *Entamoeba. E. gingivalis* is commonly found in the periodontal pockets (near the base of the tooth) of humans and pets. It is primarily found in individuals with poor oral hygiene, diabetes, immunocompromised patients, and patients with pre-existing periodontal disease. It is rarely found in people with healthy gums (overall, the amoeba is found in the gingival and dental plaque of 50% of humans). This species of *Entamoeba* is closely related to the human pathogen *Entamoeba histolytica*, the agent of amoebiasis. *E. gingivalis* may be asymptomatic in some sulci, only developing symptoms when environmental changes come about. However, several studies have not definitely demonstrated any causative correlation between *E. gingivalis* and periodontitis, making the role of this microbe in disease unknown. It appears that diseased periodontal tissue, along with an associated *Actinomyces* bacteria may provide a favorable environment for the *E. gingivalis* to develop.

Identification of *E. gingivalis* can be done by detection of trophozoites on a wet-mounted slide comprised of gum or tooth scrapings. Upon microscopic examination, their characteristic amoeboid movement can be noted. In most species of the genus *Entamoeba*, two cellular forms have been identified in nature: the cyst, which is the contaminating form found in the environment, and trophozoites, the vegetative form that are able to divide. The trophozoites, are usually 20 micrometers - 150 micrometers in diameter. They have pseudopodia that allow them to move quickly and phagocytise the nucleus of polynuclear neutrophils in periodontal disease. Their nucleus is spherical in shape and 2 - 4 micrometers in diameter, with a small, central endosome. They also contain numerous food vacuoles in the endoplasm, which consists mostly of PMN nuclei, leukocytes (especially in the case of gingival inflammation), and bacteria.

Two genetic sub types exist, the ST1 and ST2-kamaktli subtypes. The genetic variability in the species *E. gingivalis* seems important and the genetic distance between the ST1 and ST2 variants may indicate differences in their biology, which may result in different pathological manifestation.

Outside of the oral cavity, *E. gingivalis* may rarely be found in sputum or detected in cervical Pap smears. As such, it is important to differentiate them from the morphologically-similar trophozoites of *E. histolytica,* which may be found in sputum from pulmonary abscesses and which may invade the female genital tract.

Life cycle. Trophozoites initially derive from excystation of cysts ingested by the host. The survival of *Entamoeba* is ensured by their encystment in response to environmental changes, based on how they survive in an environment exposed to oxygen (ie. Feces). However, it is now commonly accepted that *E. gingivalis* does not produce cysts, nevertheless due to the fact that *E. gingivalis* are found deep in periodontal pockets, we can understand that low oxygen levels are important for survival. They do not survive intestinal passage; thus transmission can be direct from one person to another by kissing or sharing utensils (via saliva or the calculus dental plaque). However, since the complete life cycle of *E. gingivalis* is yet to be understood, we can not yet take measures to understand efficient prophylaxis.

Pathophysiology. Commonly accepted models explain the progression from healthy gums to gingivitis and then to periodontitis by a gradual change in the identity and proportion of bacterial microorganisms in the periodontal pockets. Although not pathological, inflammation is always present in periodontitis. Leukocytes are recruited to inflamed gums and their passage to the periodontal pocket lumen seem to fuel both tissue destruction and the development of the flora. Thus, maxillodental abscesses may form, especially in the case of immunocompromised patients.

During periodontitis, bacterial virulence genes are strongly modulated and the interactions between constituents of the microbiota could be essential for their functions during the pathophysiology of the disease.

The ability of *E. histolytica* to kill and phagocytose host cells correlates with parasite virulence and this amoeba is able to feed on human cells: erythrocytes, lymphocytes, and epithelial cells. Two mechanisms of cell killing and uptake have been discovered for *E. histolytica*: phagocytosis and trogocytosis. Phagocytosis is the phenomenon by which single cells ingest large volumes of material, like other cells or large particles. *Entamoeba gingivalis* is able to engulf one or more human cells at a time.

E gingivalis can trigger signals leading to modification of human cells (PMNS-neutrophils). The processes leading to the modifications in nuclear and cytoplasmic morphology in these cells remain to be defined and could be linked, for instance, to proteolytic activity of the amoebae and bacteria, or to a delayed Neutrophil extracellular traps. Whatever this process is, and whether the amoeba phagocytoses or trogocytosis, the cellular content of the neutrophils leads to one certain point: the first line of defense of cellular innate immunity against *E. gingivalis* and other organisms in the dysbiotic biofilm lacks its weapon (nuclei for NET formation and gene expression) and is thus unable to accomplish its functions.

In infected periodontal crevices, *Entamoeba gingivalis* moves and feeds on the nucleus of white blood cells. The amoeba can penetrate into the cytoplasm to suction the content of its nucleus using the negative pressure of its pseudopods. This content is then gradually absorbed into the endoplasm. Sometimes up to 20 polynuclear neutrophil nuclei can be phagocytized. This activity leaves a denucleated cell, not able to perform its proper function. Besides this, it can have a pathogenic activity by releasing uncontrolled PMN proteolytic enzymes to surrounding tissues.

Prophylaxis includes following correct oral hygiene and using clean dishes or cups. Treatment is not usually necessary if appropriate oral hygiene measures are followed.

31.2 Trichomonas tenax

Trichomonas tenax is the smallest species in the genus *Trichomonas* and is commonly found as a commensal organism in the oral cavity of animals and patients with poor oral hygiene and advanced periodontal disease (elderly patients). Routine hygiene is generally not sufficient enough to eliminate the parasite, which explains its Latin name meaning "tenacious". Transmission is through saliva (where it can survive for up to 48 hours, oral droplets, and kissing or use of contaminated dishes or drinking water.

Collection and **identification** of the parasite can be done through mouth and dental scrapings (including the tartar between the teeth and gingival margins). Specific detection can be done by polymerase chain reaction, but identification can also be performed via microscopic examination and culture media.

Microscopic examination of tonsillar crypts and pyorrheal pockets of patients suffering from *T. tenax* infections often yields trophozoites. *T. tenax* trophozoite are oval or pair shaped and only 5-14 µm long and 6-9 µm wide; specimens can be identified by their long axostyles and tails, 4 anterior flagella, and by the recurrent flagellum that raises an undulating membrane (2/3 the length of its body). This undulating membrane may appear like small legs. They have a single ovoid shaped nucleus with vesicular chromatin granules. *T. tenax* does not form cysts and is transmitted directly from the trophozoite form. In some cases, the distinction between that of an oral or vaginal parasite should be confirmed, due to the undulating membrane being larger than normal (resembling that of *T. vaginalis*) and due to the ease with which the parasite can be transmitted through direct contact of mucous membranes.

Life cycle. *T. tenax* trophozoites in the mouth are scavengers that feed primarily on local microorganisms located between the teeth, tonsillar crypts, pyorrheal pockets, and the gingival margin. They multiply by longitudinal binary fission. The trophozoites are unable to survive the digestive process.

Pathophysiology. *T. Tenax* is considered to be a commensal flora of the human mouth. When the conditions in the oral cavity are favorable for its growth and survival, *T. tenax* can be obtained in and around the diseased and necrotic teeth and gums.

In infected hosts, the parasite can typically be found in dental calculus, as well as within the tonsillar crypts, which become purulent during the course of infection. It can show proteolytic and collagenolytics activity. *T. tenax* may also be involved in the degradation of periodontal tissue through the secretion of substances such as alkaline phosphatases and the fibronectin cathepsine. *T. tenax* can can damage to host tissues, its behavior in relation with contact cells is similar to that of

T. vaginalis. T. tenax can also produce plasma membrane projections and phagocytize portions of human cells.

Alone, *T. tenax* is not known to cause any symptoms, but it is known to worsen pre-existing periodontal disease, by playing a pathogenic role in necrotizing ulcerative gingivitis and necrotizing ulcerative periodontitis. Upon aspiration from the oropharynx, *T. tenax* can cause bronchopulmonary infections, especially in patient with lung cancer or in patients with chronic purulent and necrotic purulent lung disease. In some cases, removal of the parasite is sufficient enough to allow recovery.

The dental plaque seems to be an oral structure where *T. tenax* finds the best environment for its growth and survival in the physiologic conditions of the human mouth.

Dental plaque is a very dynamic ecosystem passing through several phases in its development, starting with the initial adherence phase where microorganisms' pioneers adhere to the tooth surface (in this phase, oxygen may get into the plaque - aerobic conditions). Then, the intermediary phase follows, where an intensive struggle among microorganisms for predominance in the environment takes place (oxygen may still get into the plaque), until a relative plaque metabolism stability in a microbe community is reached (the final phase when oxygen is not available, anaerobic conditions). The final phase lasts until the occurrence of enamel cavitation, whereby the microorganisms' life basis undergoes changes. According to this, we may suppose that a mature dental plaque, the final phase of plaque development being reached, is the best life environment for anaerobic *Trichomonas tenax.*

The presence of *T. tenax* in the deep periodontal pocket may substantiate this role in periodontal dysbiosis. Besides the inflammation and the deepening of the periodontal pockets, the periodontal environment becomes more anaerobic, resulting in a bacterial shift from a Gram- positive to a Gram-negative flora. This decrease in the partial pressure in oxygen may explain why the depth of periodontal pocket may be a critical factor for the anaerobic *T. tenax* colonization and growth.

Clinical signs include gingival inflammation and bleeding, alveolar bone loss leading to periodontal pocket formation, gingival recession and, in later phases, tooth mobility and subsequent loss.

No matter the patient's state of health, it seems that oral hygiene instructions in combination with scaling can help with controlling excessive colonization of parasites, not only in the case of *T. tenax,* but also in the case of *E. gingivalis* and their probable opportunistic infestation. Prophylaxis can be brushing teeth at least 3 times daily, treatment being unnecessary in the case of good oral hygiene. The active participation of the flagellate in oral inflammatory disease has been suggested by improvement of diseases after metronidazole therapy.

31.3 Giardia lamblia

Classification. *G. lamblia* is a flagellated parasitic organism from the genus *Giardia*, that typically colonizes and reproduces in the lumen of the small intestine,

causing giardiasis. The parasite attaches to the epithelium by a ventral adhesive disc or sucker, and reproduces via binary fission. Chief pathways of human infection include ingestion of untreated sewage.

Identification. Giardiasis is diagnosed by the identification of cysts or trophozoites in feces, using direct mounts and concentration procedures. Cysts are typically seen in wet mount preparations, while trophozoites are seen in permanent mounts (trichrome staining). Repeated samples may be necessary. In addition, samples of duodenal fluid (Enterotest) or duodenal biopsy may demonstrate trophozoites. Alternate methods for detection include antigen detection tests by enzyme immunoassays, and detection of parasites by immunofluorescence. Both methods are available in commercial kits.

The trophozoite form appears as pear-shaped cells, 10 - 20 micrometers long, 7 - 10 micrometers across, and 2 - 4 micrometers thick. Their mobility is attributed to their four pairs of flagella, which propel the trophozoites through the intestine. Each cell has two nuclei, both of which actively transcribe genes. Adjacent to the nucleus, the cells have an endoplasmic reticulum that extends through much of the cell. Peripheral vesicles are responsible both for taking up extracellular nutrients, and expelling waste outside the cell. Each cell also contains a pair of rigid structures called median bodies which make up part of the cytoskeleton.Trophozoites adhere to host epithelial cells via a specialized disk-shaped organelle called the ventral disk.

Cysts are oval-shaped cells slightly smaller than trophozoites. They lack flagella, and are covered by a smooth, clear cyst wall. Each cyst contains the organelles for two trophozoites: four nuclei, two ventral disks, etc. Cysts are its most resistant form and are responsible for transmission of giardiasis.

Life cycle. Cysts are the resistant form (can survive several months in cold water) and are responsible for transmission of giardiasis. Both cysts and trophozoites can be found in feces. Infection occurs by the ingestion of cysts in contaminated water, food, or by the fecal-oral route. In the small intestine, excystation releases trophozoites (each cyst produces two trophozoites). Trophozoites multiply by longitudinal binary fission, remaining in the lumen of the proximal small bowel where they can be free or attached to the mucosa by their ventral sucking disk. Encystation occurs as the parasites transit toward the colon. The cyst is found most commonly in non-diarrheal feces. Because the cysts are infectious when passed in the stool or shortly afterward, person-to-person transmission is possible. While animals can also be infected with *Giardia*, their importance as a reservoir is unclear.

Pathophysiology. Infection does not often cause symptoms. The spectrum varies from asymptomatic carriage to severe diarrhea and malabsorption. Acute giardiasis develops after an incubation period of 1 to 14 days (average of 7 days) and usually lasts 1 to 3 weeks. Symptoms include diarrhea, abdominal pain, bloating, nausea, and vomiting. In chronic giardiasis the symptoms are recurrent and malabsorption and debilitation may occur. In immunocompromised patients it may lead to malabsorption. Finally, they can sometimes cause aphthous stomatitis (ulcers).

It is increasingly recognized that, within the luminal ecology of the human intestine, resident microbiota modifies ingested nutrients and their metabolites, influencing nutrient availability and uptake. *Giardia* lacks fundamental enzymes necessary for generating critical biomolecules such as cholesterol and therefore must rely on acquisition of these materials from the luminal environment. The parasite acquires nutrients primarily via bulk-phase uptake in endocytic vacuoles localized to the exposed dorsal (non-adherent) side adheres to epithelial cells using the ventral disc, and may direct fluid flow toward adherent parasites via coordinated flagellar motion.

Pathophysiology is believed to involve heightened rated of enterocytes apoptosis, intestinal barrier dysfunction, activation of host lymphocytes, shortening of brush border microvilli with or without coinciding villous atrophy, disaccharidase deficiencies, small intestinal malabsorption, anion hypersecretion and increased intestinal transit rates. As it is the case with other enteropathogens, induction of apoptosis in enterocytes by *Giardia* represents a key component in the pathogenesis of the infection

Prophylaxis includes good hygiene, avoid cooking potentially contaminated food, and boiling drinking water. Several drugs can be used to treat an infection with *Giardia*. Effective treatments include metronidazole, tinidazole, and nitazoxanide. Alternative treatment includes paromomycin, quinacrine, and furazolidone. Different factors may shape how effective a drug regimen will be for a patient, including their medical history, nutritional status, and immune status.

32. BASIC VIROLOGY

In contrast to bacteria, fungi, and parasites, viruses are *not* cells, ie, they are not capable of reproducing independently, do not have a nucleus, and do not have organelles such as ribosomes, mitochondria, and lysosomes. Viruses are smaller than cells and cannot be seen in the light microscope.

(1) Viruses are particles composed of an internal core containing *either* DNA *or* RNA (but not both) covered by a protective protein coat. Some viruses have an outer lipoprotein membrane, called an envelope, external to the coat.

(2) Viruses must reproduce (replicate) within cells, because they cannot generate energy or synthesize proteins. Because they can reproduce only within cells, viruses are "obligate intracellular parasites."

(3) Viruses replicate in a manner different from that of cells; ie, viruses do not undergo binary fission or mitosis.

Table 14 compares some of the attributes of viruses and cells.

Table 14. Comparison of viruses and cells.

Property	**Viruses**	**Cells**
Type of nucleic acid	DNA or RNA but not both	DNA and RNA
Proteins	Few	Many
Lipoprotein membrane	Envelope present in some viruses	Cell membrane present in all cells
Ribosomes	Absent'	Present
Mitochondria	Absent	Present in eukaryotic
Enzymes	None or few	Many
Multiplication by binary fission or mitosis	No	Yes

Arenaviruses have a few nonfunctional ribosomes.

Structure

SIZE & SHAPE

Viruses range from 20 to 300 nm in diameter; this corresponds roughly to a range of sizes from that of the largest protein to that of the smallest cell. Their shapes are frequently referred to in colloquial terms, eg, spheres, rods, bullets, or bricks, but in reality they are complex structures of precise geometric symmetry (see below). The shape of virus particles is determined by the arrangement of the repeating subunits that form the protein coat (capsid) of the virus.

VIRAL NUCLEIC ACIDS

The viral nucleic acid (genome) is located internally and can be either single- or double-stranded DNA or single- or doublestranded RNA. Only viruses have genetic

material composed of single-stranded DNA or of singlestranded or double-stranded RNA. The nucleic acid can be either linear or circular. The DNA is always a single molecule; the RNA can exist either as a single molecule or in several pieces. Almost all viruses contain only a single copy of their genome; ie, they are haploid. The exception is the retrovirus family, whose members have two copies of their RNA genome; ie, they are diploid.

CAPSID & SYMMETRY

The nucleic acid is surrounded by a protein coat called a capsid, made up of subunits called capsomers. Each capsomer, consisting of one or several proteins, can be seen in the electron microscope as a spherical particle, sometimes with a central hole. The arrangement of capsomers gives the virus structure its geometric symmetry. There are two forms of symmetry in virus capsids: (1) icosahedral, in which the capsomers are arranged in 20 triangles that form a symmetric figure (an icosahedron) with the approximate outline of a sphere; and (2) helical, in which the capsomers are arranged in a hollow coil that appears rod-shaped. The helix can be either rigid or flexible. Both the icosahedral and the helical forms can exist either as a "naked" nucleocapsid or with an outer envelope layer.

The advantage of building the virus particle from identical protein subunits is 2-fold: (1) it reduces the need for genetic information, and (2) it promotes self-assembly; ie, no enzyme or energy is required. In fact, functional virus particles have been assembled in the test tube by combining the purified nucleic acid with the purified proteins in the absence of cells, energy source, and enzymes.

VIRAL PROTEINS

Viral proteins serve several important functions. The outer capsid proteins protect the genetic material and mediate the attachment of the virus to specific receptors on the host cell surface. This interaction of the viral proteins with the cell receptor is the major determinant of species and organ specificity. Outer viral proteins are also important antigens that induce neutralizing antibody and activate cytotoxic T cells to kill virus-infected cells. The outer proteins induce these immune responses following both the natural infection and immunization (see below). Some of the internal proteins are associated with the nucleic acid, eg, nucleic acid polymerases, which are essential for replication. Histonelike proteins, which may have a regulatory function or may neutralize the negative charge on the nucleic acid during assembly of the virus particle, are also located internally.

ENVELOPE

In addition to the capsid and internal proteins, there are two other types of proteins, both of which are associated with the envelope. The envelope is a lipoprotein membrane composed of lipid derived from the host cell membrane and protein that is virus-specific. Furthermore, there are frequently glycoproteins in the form of spikelike projections on the surface, which attach to host cell receptors during

the entry of the virus into the cell. Another protein, the matrix protein, mediates the interaction between the capsid proteins and the envelope.

In general, the presence of an envelope confers instability on the virus. Enveloped viruses are more sensitive to heat, detergents, and lipid solvents such as alcohol and ether than are nonenveloped (nucleocapsid) viruses, which are composed only of nucleic acid and capsid proteins.

The surface proteins of the virus, whether they are the capsid proteins or the envelope glycoproteins, are the principal antigens against which the host mounts its immune response to viruses. They are also the determinants of type specificity. For example, poliovirus types 1, 2, and 3 are distinguished by the antigenicity of their capsid proteins. It is important to know the number of serotypes of a virus, since vaccines should contain the prevalent serotypes. There is often little cross protection between different serotypes.

ATYPICAL VIRUSLIKE AGENTS

There are four exceptions to the typical virus as described above:

(1) Defective viruses are composed of viral nucleic acid and proteins but cannot replicate without a "helper" virus, which provides the missing function. Defective viruses usually have a mutation or a deletion of part of their genetic material. During the growth of most human viruses, many more defective than infectious virus particles are produced. The ratio of defective to infectious particles can be as high as 100:1. Because these defective particles can interfere with the growth of the infectious particles, it has been hypothesized that the defective viruses may aid in recovery from an infection by limiting the ability of the infectious particles to grow.

(2) Pseudovirions contain host cell DNA instead of viral DNA within the capsid. They are formed during infection with certain viruses when the host cell DNA is fragmented and pieces of it are incorporated within the capsid protein. Pseudovirions can infect cells, but they do not replicate.

(3) Viroids consist solely of a single molecule of circular RNA without a protein coat or envelope. There is extensive homology between bases in the viroid RNA, leading to large double-stranded regions. The RNA is quite small (MW 1 x 10') and apparently does not code for any protein. Nevertheless, viroids replicate but the mechanism is unclear. They cause several plant diseases but are not implicated in any human disease.

(4) Prions are infectious protein particles that are composed solely of protein; ie, they contain no detectable nucleic acid. They are implicated as the cause of certain "slow" diseases such as CreutzfeldtJakob disease in humans and scrapie in sheep. Since neither DNA nor RNA has been detected in prions, they are clearly different from viruses. Furthermore, electron microscopy reveals filaments rather than virus particles. Prions are much more resistant to inactivation by ultraviolet light and heat than are viruses. They are remarkably resistant to formaldehyde and nucleases. However, they are inactivated by hypochlorite, NaOH, and autoclaving. Hypochlorite

is used to sterilize surgical instruments and other medical supplies that cannot be autoclaved.

Table 15. Comparison of prions and conventional viruses.

Feature	Prions	Conventional Viruses
Particle contains nucleic acid	No	Yes
Particle contains protein	Yes, encoded by cellular	Yes, encoded by viral
Inactivated rapidly by UV light or heat	No	Yes
Appearance in electron microscope	Filamentous rods (amyloid-like)	Icosahedral or helical symmetry
Infection induces antibody	No	Yes
Infection induces inflammation	No	Yes

Prions are composed of a single glycoprotein with a molecular weight of 27,000-30,000. With scrapie prions as the model, it was found that this protein is encoded by a single cellular gene. This gene is found in equal numbers in the cells of both infected and uninfected animals. Furthermore, the amount of prion protein mRNA is the same in uninfected as in infected cells. In view of these findings, posttranslational modifications of the prion protein are hypothesized to be the important distinction between the protein found in infected and uninfected cells. There is evidence that a change in the conformation from the normal alpha-helical form to the abnormal beta-pleated sheet form is the important modification. The abnormal form then recruits additional normal forms to change their configuration, and the number of abnormal pathogenic particles increases. The prion protein in normal cells is protease-sensitive, whereas the prion protein in infected cells is protease-resistant, probably because of the change in configuration.

The function of the normal prion protein is unknown. "Knockout" mice in which the gene encoding the prion protein is inactive appear normal. There is some evidence that it is a copper-binding protein.

The observation that the prion protein is the product of a normal cellular gene may explain why no immune response is formed against this protein; ie, tolerance occurs. Similarly, there is no inflammatory response in infected brain tissue. A vacuolated (spongiform) appearance is found, without inflammatory cells. Prion proteins in infected brain tissue form rod-shaped particles that are morphologically and histochemically indistinguishable from amyloid, a substance found in the brain tissue of individuals with various central nervous system diseases (as well as diseases of other organs).

The role of prions in the pathogenesis of "slow" diseases such as Creutzfeldt-Jakob disease remains unclear. The central question is: are prions the cause of these

diseases or a pathologic byproduct? At present, prions are the most plausible causative agents and no alternative explanation has gathered much support.

Replication

The viral replication cycle is described below in two different ways. The first approach is a growth curve, which shows the amount of virus produced at different times after infection. The second is a stepwise description of the specific events within the cell during virus growth.

VIRAL GROWTH CURVE

The amount of virus produced is plotted on a logarithmic scale as a function of time after infection. Note that the time required for the growth cycle varies; it is minutes for some bacterial viruses and hours for some human viruses.

The first event is quite striking: the virus disappears, as represented by the solid line dropping to the X axis. Although the virus particle, as such, is no longer present, the viral nucleic acid continues to function and begins to accumulate within the cell, as indicated by the dotted line. The time during which no virus is found inside the cell is known as the eclipse period. The eclipse period ends with the appearance of virus (solid line). The latent period, in contrast, is defined as the time from the onset of infection to the appearance of virus extracellularly. Note that infection begins with one virus particle and ends with several hundred virus particles having been produced; this type of reproduction is unique to viruses. Alterations of cell morphology accompanied by marked derangement of cell function begin toward the end of the latent period. This cytopathic effect (CPE) culminates in the lysis and death of cells. Not all viruses cause CPE; some can replicate while causing little morphologic or functional change in the cell.

<u>Stages of the viral growth cycle:</u>

1. Attachment and penetration by parental virion
2. Uncoating of the viral genome
3. Early viral mRNA synthesise ("Early" is defined as the period before genome replication. Not all viruses exhibit a distinction between early and late functions. In general, early proteins are enzymes, whereas late proteins are structural components of the virus. In some cases, the viral genome is functionally equivalent to mRNA; thus, early mRNA need not be synthesized.)
4. Early-viral protein synthesis
5. Viral genome replication
6. Late viral mRNA synthesis
7. Late viral protein synthesis
8. Progeny virion assembly
9. Virion release from cell

SPECIFIC EVENTS DURING THE GROWTH CYCLE

The infecting parental virus particle attaches to the cell membrane and then penetrates the host cell. The viral genome is "uncoated" by removing the capsid proteins, and the genome is free to function. Early mRNA and proteins are synthesized; the early proteins are enzymes used to replicate the viral genome. Late mRNA and proteins are then synthesized. These late proteins are the structural, capsid proteins. The progeny virions are assembled from the replicated genetic material and newly made capsid proteins and are then released from the cell.

Another, more general way to describe the growth cycle is as follows: (1) early events, ie, attachment, penetration, and uncoating; (2) middle events, ie, gene expression and genome replication; and (3) late events, ie, assembly and release. With this sequence in mind, each stage will be described in more detail.

Attachment, Penetration, & Uncoating

The proteins on the surface of the virion attach to specific receptor proteins on the cell surface through weak, noncovalent bonding. The specificity of attachment determines the host range of the virus. Some viruses have a narrow range, whereas others have quite a broad range. For example, poliovirus can enter the cells of only humans and other primates, whereas rabies virus can enter all mammalian cells. The organ specificity of viruses is governed by receptor interaction as well. Those cellular receptors that have been identified are surface proteins that serve various other functions. For example, herpes simplex virus type 1 attaches to the fibroblast growth factor receptor, rabies virus to the acetylcholine receptor, and human immunodeficiency virus to the CD4 protein on helper T lymphocytes.

The virus particle penetrates by being engulfed in a pinocytotic vesicle, within which the process of uncoating begins. A low pH within the vesicle favors uncoating. Rupture of the vesicle or fusion of the outer layer of virus with the vesicle membrane deposits the inner core of the virus into the cytoplasm.

Certain bacterial viruses (bacteriophages) have a special mechanism for entering bacteria that has no counterpart in either human viruses or those of animals or plants. Some of the T group of bacteriophages infect *Escherichia coli* by attaching several tail fibers to the cell surface and then using lysozyme from the tail to degrade a portion of the cell wall. At this point, the tail sheath contracts, driving the tip of the core through the cell wall. The viral DNA then enters the cell through the tail core, while the capsid proteins remain outside.

It is appropriate at this point to describe the phenomenon of infectious nucleic acid, since it provides a transition between the concepts of host specificity described above and early genome functioning, which is discussed below. Note that we are discussing whether the purified genome is infectious. All viruses are "infectious" in a person or in cell culture, but not all purified genomes are infectious.

Infectious nucleic acid is purified viral DNA or RNA (without any protein) that can carry out the entire viral growth cycle and result in the production of complete virus particles. This is interesting from three points of view:

(1) The observation that purified nucleic acid is infectious is the definitive proof that nucleic acid, not protein, is the genetic material.

(2) Infectious nucleic acid can bypass the host range specificity provided by the viral protein-cell receptor interaction. For example, although intact poliovirus can grow only in primate cells, purified poliovirus RNA can enter nonprimate cells, go through its usual growth cycle, and produce normal poliovirus. The poliovirus produced in the nonprimate cells can infect only primate cells, since it now has its capsid proteins. These observations indicate that the internal functions of the nonprimate cells are capable of supporting viral growth once entry has occurred.

(3) Only certain viruses yield infectious nucleic acid. The reason for this is discussed below. Note that all viruses are infectious but not all purified viral DNA's or RNA's (genomes) are infectious.

Gene Expression & Genome Replication

The first step in viral gene expression is mRNA synthesis. It is at this point that viruses follow different pathways depending on the nature of their nucleic acid and the part of the cell in which they replicate.

DNA viruses, with one exception, replicate in the nucleus and use the host cell DNA-dependent RNA polymerase to synthesize their mRNA. The poxviruses are the exception because they replicate in the cytoplasm, where they do not have access to the host cell RNA polymerase: They therefore carry their own polymerase within the virus particle. The genome of all DNA viruses consists of double-stranded DNA, except for the parvoviruses, which have a single-stranded DNA genome.

Most RNA viruses undergo their entire replicative cycle in the cytoplasm. The two principal exceptions are retroviruses and influenza viruses, both of which have an important replicative step in the nucleus. Retroviruses integrate a DNA copy of their genome into the host cell DNA, and influenza viruses synthesize their progeny genomes in the nucleus.

RNA viruses fall into four groups with quite different strategies for synthesizing mRNA.

(1) The simplest strategy is illustrated by poliovirus, which has single-stranded RNA of positive polarity as its genetic material. These viruses use their RNA genome directly as mRNA. Positive polarity is defined as an RNA with the same base sequence as the mRNA. RNA with negative polarity has a base sequence that is complementary to the mRNA. For example, if the mRNA sequence is a A-C-U-G, an RNA with negative polarity would be U-G-A-C and an RNA with positive polarity would be A-C-U-G.

(2) The second group has single-stranded RNA of negative polarity as its genetic material. An mRNA must be transcribed by using the negative strand as a template. Because the cell does not have an RNA polymerase capable of using RNA as a template, the virus carries its own RNA-dependent RNA polymerase. There are two subcategories of negative-polarity RNA viruses: those that have a single piece of RNA, eg, measles virus (a paramyxovirus) or rabies virus (a rhabdovirus), and those that have multiple pieces of RNA, eg, influenza virus (a myxovirus).

Table 16. Important features of DNA viruses.

DNA Genome	Location of Replication	Virion Polymeras	Infectivity of	Prototype Human Virus
Single strand	Nucleus	No[1,2]	Yes	Parvovirus B19
Double strand				
Circular	Nucleus	No[1]	Yes	Papillomavirus
Circular; partially single	Nucleus	Yes[3]	No	Hepatitis B virus
Linear	Nucleus	No[1]	Yes	Herpesvirus, adenovirus
Linear	Cytoplasm	Yes	No	Smallpox virus, vaccinia virus

[1] mRNA is synthesized by host cell RNA polymerase in the nucleus

[2] Single-stranded genome DNA is converted to double-stranded DNA by host cell polymerase. A virus-encoded DNA polymerase then synthesizes progeny DNA.

[3] Hepatitis B virus uses a virion-encoded RNA-dependent DNA polymerase to synthesize its progeny DNA with full-length mRNA as the template. This enzyme is a type of "reverse transcriptase" but functions at a different stage in the replicative cycle than does the reverse transcriptase of retroviruses.

Note: All DNA viruses encode their own DNA polymerase that replicates the genome. They do not use the host cell DNA polymerase (with the minor exception of the parvoviruses as mentioned above).

Table 17. Important features of RNA viruses.

RNA Genome	Polarity	Virlon Rolymeras	Source of mRNA	Infectivity of	Prototype Human Virus
Single strand,	+	No	Genome	Yes	Poliovirus
Single strand,					
Nonsegmented	-	Yes	Transcription	No	Measles virus, rabies
Segmented	-	Yes	Transcriptio	No	Influenza virus
Double strand,	±	Yes	Transcriptio	No	Rotavirus
Single strand, diploid	+	Yes[1]	Transcriptio	No[3]	HTLV, HIV[4]

[1]Retroviruses contain an RNA-dependent DNA polymerase.

[2] mRNA transcribed from DNA intermediate.

[3] Although the retroviral genome RNA is not infectious, the DNA intermediate is.

[4] HTLV, human T cell leukemia virus; HIV, human immunodeficiency virus.

(3) The third group has double-stranded RNA as its genetic material. Because the cell has no enzyme capable of transcribing this RNA into mRNA, the virus carries its own polymerase. Reovirus, the best-studied member of this group, has 10 segments of double-stranded RNA.

(4) The fourth group, exemplified by retroviruses, has single-stranded RNA of positive polarity that is transcribed into double-stranded DNA by the RNA-dependent

DNA polymerase (reverse transcriptase) carried by the virus. This DNA copy is then transcribed into viral mRNA by the regular host cell RNA polymerase (polymerase II). Retroviruses are the only family of viruses that are "diploid," ie, that have two copies of their genome RNA.

These differences explain why some viruses yield infectious nucleic acid and others do not. Viruses that do not require a polymerase in the virion can produce infectious DNA or RNA. By contrast, viruses such as the poxviruses, the negative-stranded RNA viruses, the double-stranded RNA viruses, and the retroviruses, which require a virion polymerase, cannot yield infectious nucleic acid. Several additional features of viral mRNA are described in the box.

Once the viral mRNA of either DNA or RNA viruses is synthesized, it is translated by host cell ribosomes into viral proteins, some of which are "early" proteins, ie, enzymes required for replication of the viral genome, and others are "late proteins", ie, structural proteins of the progeny viruses. (The term "early" is defined as occurring before the replication of the genome, and "late" is defined as occurring after genome replication.) The most important of the early proteins for many RNA viruses is the polymerase that will synthesize many copies of viral genetic material for the progeny virus particles. No matter how a virus makes its mRNA, virtually all viruses make a virus-encoded polymerase (a replicase) that replicates the genome, ie, that makes many copies of the parental genome that will become the genome of the progeny virions.

Some viral mRNAs are translated into precursor polypeptides that must be cleaved by proteases to produce the functional structural proteins (Fig 29-4), whereas other viral mRNAs are translated directly into structural proteins. A striking example of the former occurs during the replication of picomaviruses (eg, poliovirus, rhinovirus, and hepatitis A virus), in which the genome RNA, acting as mRNA, is translated into a single polypeptide; which is then cleaved by a virus-coded protease into various proteins. This protease is one of the proteins in the single^{-} polypeptide, an interesting example of a protease acting upon its own polypeptide.

Another important family of viruses in which precursor polypeptides are synthesized is the retrovirus family. For example, the gag, poi, and *env* genes of human immunodeficiency virus are translated into precursor polypeptides, which are then cleaved by a virus-encoded protease. It is this protease that is inhibited by the drugs classified as protease inhibitors. Flaviviruses, such as hepatitis C virus and yellow fever virus, also synthesize precursor polypeptides that must be cleaved to form functional proteins. In contrast, other viruses, such as influenza virus and rotavirus, have segmented genomes, and each segment encodes a specific functional polypeptide rather than a precursor polypeptide.

Replication of the viral genome is governed by the principle of complementarity, which requires that a strand with a complementary base sequence be synthesized; this strand then serves as the template for the synthesis of the actual viral genome.

Table 18. Complementarity in viral genome replication.

Prototype Virus	Parental Genome[1]	Intermediate Form	Progeny Genome
Poliovirus	+ ssRNA	- ssRNA	+ ssRNA
Influenza virus, measles virus, rabies virus	- ssRNA	+ ssRNA	- ssRNA
Rotavirus	dsRNA	+ ssRNA	dsRNA
Retrovirus	+ ssRNA	dsDNA	+ ssRNA
Parvovirus B19	ssDNA	dsDNA	ssDNA
Hepatitis B virus	dsDNA	+ ssRNA	dsDNA
Papovavirus, adeno-virus, herpesvirus, poxvirus	dsDNA	dsDNA	dsDNA

(1) poliovirus makes a negative-strand intermediate, which is the template for the positive-strand genome; (2) influenza, measles, and rabies viruses make a positive-strand intermediate, which is the template for the negative-strand genome; (3) reovirus makes a positive strasnd that acts both as mRNA and as the template for the negative strand in the double-stranded genome RNA; (4) retroviruses use the negative strand of the DNA intermediate to make positive-strand progeny RNA; (5) hepatitis B virus uses its mRNA as a template to make progeny double-stranded DNA; and (6) the other double-stranded DNA viruses replicate their DNA by the same semiconservative process by which cell DNA is synthesized.

As the replication of the viral genome proceeds, the structural capsid proteins to be used in the progeny virus particles are synthesized. In some cases, the newly replicated viral genomes can serve as templates for the late mRNA to make these capsid proteins.

Viral mRNA

There are four interesting aspects of viral mRNA and its expression in eukaryotic cells. (1) Viral mRNAs have three attributes in common with cellular mRNAs: on the 5' end there is a methylated GTP "cap", which is linked by an "inverted" (3'-to-5') bond instead of the usual 5'to-3' bond; on the 3' end there is a tail of 100-200 adenosine residues [poly(A)]; and the mRNA is generated by splicing from a larger transcript of the genome. In fact, these three modifications were first observed in studies on viral mRNAs and then extended to cellular mRNAs. (2) Some viruses use their genetic material to the fullest extent by making more than one type of mRNA from the same piece of DNA by "shifting the reading frame." This is done by starting transcription 1 or 2 bases downstream from the original initiation site. (3) With some DNA viruses, there is temporal control over the region of the genome that is transcribed into mRNA. During the beginning stages of the growth cycle, before DNA replication begins, only the early region of the genome is transcribed and, therefore, only certain early proteins are made. One of the early proteins is a

repressor of the late genes; this prevents transcription until the appropriate time. (4) Three different processes are used to generate the monocistronic mRNAs that will code for a single protein from the polycistronic viral genome:

(1) Individual mRNAs are transcribed by starting at many specific initiation points along the genome, which is the same mechanism used by eukaryotic cells and by herpesviruses, adenoviruses, and the DNA and RNA tumor viruses;

(2) in the reoviruses and influenza viruses, the genome is segmented into multiple pieces, each of which codes for a single mRNA; and

(3) in polioviruses, the entire RNA genome is translated into one long polypeptide, which is then cleaved into specific proteins by a protease.

Assembly & Release

The progeny particles are assembled by packaging the viral nucleic acid within the capsid proteins. Little is known about the precise steps in the assembly process. Surprisingly, certain viruses can be assembled in the test tube by using only purified RNA and purified protein. This indicates that the specificity of the interaction resides within the RNA and protein and that the action of enzymes and expenditure of energy are not required.

Virus particles are released from the cell by either of two processes. One is rupture of the cell membrane and release of the mature particles; this usually occurs with unenveloped viruses. The other, which occurs with enveloped viruses, is release of viruses by "budding" through the outer cell membrane. (An exception is the herpesvirus family, whose members acquire their envelopes from the nuclear membrane rather than from the outer cell membrane.) The budding process begins when virus-specific proteins enter the cell membrane at specific sites. The viral nucleocapsid then interacts with the specific membrane site mediated by the matrix protein. The cell membrane evaginates at that site, and an enveloped particle buds off from the membrane. Budding frequently does not damage the cell, and in certain instances the cell survives while producing large numbers of budding virus particles.

LYSOGENY

The typical replicative cycle described above occurs most of the time when viruses infect cells. However, some viruses can use an alternative pathway, called the lysogenic cycle, in which the viral DNA becomes integrated into the host cell chromosome and no progeny virus particles are produced at that time. The viral nucleic acid continues to function in the integrated state in a variety of ways. One of the most important functions from a medical point of view is the synthesis of several exotoxins in bacteria, such as diphtheria, botulinum, cholera, and erythrogenic toxins, coded for by the genes of the integrated bacteriophage (prophage). Lysogenic conversion is the term applied to the new properties that a bacterium acquires as a result of expression of the integrated prophage genes.

The lysogenic or "temperate" cycle is described for lambda bacteriophage, because it is the bestunderstood model system. Several aspects of infections by tumor viruses and herpesviruses are similar to the events in the lysogenic cycle of lambda phage.

Infection by lambda phage in *E coli* begins with injection of the linear, double-stranded DNA genome through the phage tail into the cell. The linear DNA becomes a circle as the single-stranded regions on the 5' end and the 3' end pair their complementary bases. A ligating enzyme makes a covalent bond in each strand to close the circle. Circularization is important, because it is the circular form that integrates into host cell DNA.

The choice between the pathway leading to lysogeny and that leading to full replication is made as early protein synthesis begins. Simply put, the choice depends on the balance between two proteins, the repressor produced by the c-I gene and the antagonizer of the repressor produced by the cro gene. If the repressor predominates, transcription of other early genes is shut off and lysogeny ensues. Transcription is inhibited by binding of the repressor to the two operator sites that control early protein synthesis. If the cro gene product prevents the synthesis of sufficient repressor, replication and lysis of the cell result. One correlate of the lysogenic state is that the repressor can also prevent the replication of additional lambda phages that infect subsequently. This is called "immunity" and is specifically directed against lambda phage, because the repressor binds only to the operator sites in lambda DNA; other phages are not affected.

The next important step in the lysogenic cycle is the integration of the viral DNA into the cell DNA. This occurs by the matching of a specific attachment site on the lambda DNA to a homologous site on the *E coli* DNA and the integration (breakage and rejoining) of the two DNAs mediated by a phage-encoded recombination enzyme. The integrated viral DNA is called a prophage. Most lysogenic phages integrate at one or a few specific sites, but some, such as the Mu (or mutator) phage, can integrate their DNA at many sites, and other phages, such as the P1 phage, never actually integrate but remain in a "temperate" state extrachromosomally, similar to a plasmid.

Because the integrated viral DNA is replicated along with the,cell DNA, each daughter cell inherits a copy. However, the prophage is not permanently integrated. It can be induced to resume its replicative cycle by the action of UV light and certain chemicals that damage DNA. UV light induces the synthesis of a protease, which cleaves the repressor. Early genes then function, including the genes coding for the enzymes that excise the prophage from the cell DNA. The virus then completes its replicative cycle, leading to the production of progeny virus and lysis of the cell.

Genetics & Gene Therapy

The study of viral genetics falls into two general areas: (1) mutations and their effect on replication and pathogenesis, and (2) the interaction of two genetically distinct viruses that infect the same cell. In addition, viruses serve as vectors in gene therapy and in recombinant vaccines, two areas that hold great promise for the treatment of genetic diseases and the prevention of infectious diseases.

MUTATIONS

Mutations in viral DNA and RNA occur by the same processes of base substitution, deletion, and frame shift as those described for bacteria in Chapter 4.

Probably the most important practical use of mutations is in the production of vaccines containing live, attenuated virus. These attenuated mutants have lost their pathogenicity but have retained their antigenicity, so that they induce immunity without causing disease.

Conditional-lethal mutations are extremely valuable in determining the function of viral genes. These mutations function normally under permissive conditions but fail to replicate or to express the mutant gene under restrictive conditions. For example, temperature-sensitive conditional-lethal mutants express their phenotype normally at a low (permissive) temperature, but at a higher (restrictive) temperature the mutant gene product is inactive. To give a specific example, temperature-sensitive mutants of Rous sarcoma virus can transform cells to malignancy at the permissive temperature of 37 °C. When the transformed cells are grown at the restrictive temperature of 41 °C, their phenotype reverts to normal appearance and behavior. The malignant phenotype is regained when the permissive temperature is restored. Temperature-sensitive mutants of influenza virus can be used to make a vaccine, because this virus will grow in the cooler, upper airways where it causes few symptoms and induces antibodies but will not grow in the warmer, lower airways where it can cause pneumonia.

Some deletion mutants have the unusual property of being defective interfering particles. They are defective because they cannot replicate unless the deleted function is supplied by a "helper" virus. They also interfere with the growth of normal virus if they infect first and preempt the required cellular functions. Defective interfering particles may play a role in recovery from viral infection; they interfere with the production of progeny virus, thereby limiting the spread of the virus to other cells.

There are two other kinds of mutants of interest. The first are antigenic variants such as those that occur frequently with influenza viruses, which have an altered surface protein and are therefore no longer inhibited by a person's preexisting antibody. The variant can thus cause disease, whereas the original strain cannot. The second are drug-resistant mutants, which are insensitive to an antiviral drug because the target of the drug, usually a viral enzyme, has been modified.

INTERACTIONS

When two genetically distinct viruses infect a cell, three different phenomena can ensue.

(1) Recombination is the exchange of genes between two chromosomes that is based on crossing over within regions of significant base sequence homology. Recombination can be readily demonstrated for viruses with double-stranded DNA as the genetic material and has been used to determine their genetic map. However, recombination by RNA viruses occurs at a very low frequency, if at all. Reassortment is the term used when viruses with segmented genomes, such as influenza virus, exchange segments. This usually results in a much higher frequency of gene exchange than does recombination. Reassortment of influenza virus RNA segments is involved

in the major antigenic changes in the virus that are the basis for recurrent influenza epidemics.

(2) Complementation can occur when either one or both of the two viruses that infect the cell have a mutation that results in a nonfunctional protein (Fig 30-1). The nonmutated virus "complements" the mutated one by making a functional protein that serves for both viruses. Complementation is an important method by which a helper virus permits replication of a defective virus. One clinically important example of complementation: is hepatitis B virus providing its surface antigen to hepatitis delta virus which is defective in its ability to produce its own outer protein.

This phenomenon is the basis for the complementation test, which can be used to determine how many genes exist in a viral genome. It is performed by determining whether mutant virus A can complement mutant virus B. If it can, the two mutations are in separate genes because they make different, complementary proteins. If it cannot, the two mutations are in the same gene and both proteins are nonfunctional. By performing many of these paired tests with different mutants, it is possible to determine functional domains of complementation groups that correspond to genes. Appropriate controls are needed to obviate the effects of recombination.

(3) In phenotypic mixing, the genome of virus type A can be coated with the surface proteins of virus type B. This phenotypically mixed virus can infect cells as determined by its type B protein coat. However, the progeny virus from this infection has a type A coat; it is encoded solely by its type A genetic material. An interesting example of phenotypic mixing is that of pseudotypes, which consist of the nucleocapsid of one virus and the envelope of another. Pseudotypes composed of the nucleocapsid of vesicular stomatitis virus (a rhabdovirus) and the envelope of human immunodeficiency virus (HIV, a retrovirus) are currently being used to study the immune response to HIV.

GENE THERAPY & RECOMBINANT VACCINES

Viruses are being used as genetic vectors in two novel ways: (1) to deliver new, functional genes to patients with genetic diseases (gene therapy): and (2) to produce new viral vaccines that contain recombinant viruses carrying the genes of several different viruses, thereby inducing immunity to several diseases with one immunization.

Gene Therapy Retroviruses are currently being used as vectors of the gene encoding adenine deaminase (ADA) in patients with immunodeficiencies resulting from a defective ADA gene. Retroviruses are excellent vectors because a DNA copy of their RNA genome is stably integrated into the host cell DNA and the integrated genes are expressed efficiently. Retroviral vectors are constructed by removing the genes encoding several viral proteins from the virus and replacing them with the human gene of interest, eg, the ADA gene. Virus particles containing the human gene are produced within "helper cells" that contain the deleted viral genes and therefore can supply, by complementation, the missing viral proteins necessary for the virus to

replicate. The retroviruses produced by the helper cells can infect the patient's cells and introduce the human gene into the cells, but the viruses -cannot replicate because they lack several viral genes. This inability of these viruses to replicate is an important advantage in human gene therapy.

Recombinant Vaccines Recombinant viral, vaccines contain viruses that have been genetically engineered to carry the genes of other viruses Viruses with large genomes, eg, vaccinia virus, are excellent candidates for this purpose. To construct the recombinant virus, any vaccinia virus gene that is not essential for viral replication is deleted and the gene from the other virus that encodes the antigen which elicits neutralizing antibody is introduced. For example, the gene for the surface antigen of hepatitis B virus has been introduced into vaccinia virus and is expressed in infected cells. Recombinant vaccines are not yet clinically available, but vaccines of this type promise to greatly improve the efficiency of our immunization programs

Classification of Medically Important Viruses

The classification of viruses is based on chemical and morphologic criteria. The two major components of the virus used in classification are (1) the nucleic acid (its molecular weight and structure); and (2) the capsid (its size and symmetry and whether it is enveloped). This scheme was simplified from the complete classification to emphasize organisms of medical importance. Only the virus families are listed; subfamilies are described in the chapter on the specific virus.

DNA VIRUSES

The three naked (ie, nonenveloped) icosahedral virus families – the parvoviruses, papovaviruses, and adenoviruses-are presented in order of increasing particle size, as are the three enveloped families. The hepadnavirus family, which includes hepatitis B virus, and the herpesviruses are enveloped icosahedral viruses. The largest viruses, the poxviruses, have a complex internal symmetry.

Parvoviruses

These are very small (22 nm in diameter), naked icosahedral viruses with singlestranded linear DNA. There are two types of parvoviruses: defective and nondefective. The defective parvoviruses, eg, adeno-associated virus, require a helper virus for replication. The DNA of defective parvoviruses is unusual, because plus-strand DNA and minus-strand DNA are carried in separate particles. The nondefective parvoviruses are best illustrated by B 19 virus, which is associated with aplastic crises in sickle cell anemia patients and with erythema infectiosum, an innocuous childhood disease characterized by a "slapped-cheeks" rash.

Papovaviruses

These are naked icosahedral viruses (55 nm in diameter) with double-stranded circular supercoiled DNA. The name "papova" is an acronym of papilloma, polyoma, and simian vacuolating viruses. Three human papovaviruses are JC virus, isolated from patients with progressive multifocal leukoencephalopathy; BK virus, isolated from the urine of immunosuppressed kidney transplant patients; and human

papillomavirus. Polyomavirus and simian vacuolating virus 40 (SV40 virus) are papovaviruses of mice and monkeys, respectively, that induce malignant tumors in a variety of species.

Table 19. Classification of DNA viruses.

Virus	Envelope Present	Capsid Symmetry	Particle Size (nm)	DNA MW (x 10^6)	DNA Structure[1]	Medically Important Viruses
Parvovirus	No	Icosahedr	22	2	SS, linear	B19 virus
Papovavir	No	Icosahedr	55	3-5	DS, circular; supercoiled	Papillomavirus
Adenoviru	No	Icosahedr	75	23	DS, linear	Adenovirus
Hepadnavi	Yes	fcosahedr	42	1.5	DS, circular	Hepatitis B virus
Herpesvir	Yes	Icosahedr	100[2]	100-150	DS, linear	Herpes simplex variceila-zoster virus, cytomegalovirus: Epstein-Barr virus
Poxvirus	Yes	Complex	250 x	125-185	DS; linear	Smallpox virus, vaccinia virus

[1] SS, single-stranded; DS, double-stranded.

[2] The herpesvirus nucleocapsid is 100 nm, but the envelope varies in size. The entire virus can' be as large as 200 nm in diameter.

Adenoviruses

These are naked icosahedral viruses (75 nm in diameter) with double-stranded linear DNA. They cause pharyngitis, upper and lower respiratory tract disease, and a variety of other less common infections. There are at least 40 antigenic types, some of which cause sarcomas in animals but no tumors in humans.

Hepadnaviruses

These are double-shelled viruses (42 nm in diameter) with an icosahedral capsid covered by an envelope. The DNA is a double-stranded circle that is unusual because the complete strand is not a covalently closed circle and the other strand is missing approximately 25% of its length. Hepatitis B virus is the human pathogen in this family.

Herpesviruses

These are enveloped viruses (100 nm in diameter) with an icosahedral nucleocapsid and double-stranded linear DNA. They are noted for causing latent infections. The five important human pathogens are herpes simplex virus types 1 and 2, varicella-zoster virus, cytomegalovirus, and Epstein-Barr virus (the cause of infectious mononucleosis).

Poxviruses

These are the largest viruses, with a bricklike shape, an envelope with an unusual appearance, and a complex capsid symmetry. They are named for the skin lesions, or "pocks," that they cause. Smallpox virus and vaccinia virus are the two important members. The latter virus is used in the smallpox vaccine.

RNA VIRUSES

The three naked icosahedral virus families are listed first and are followed by the three enveloped icosahedral viruses. The remaining eight families are enveloped helical viruses; the first five have single-stranded linear RNA as their genome, whereas the last three have single-stranded circular RNA.

Picornaviruses

These are the smallest (28 nm in diameter) RNA viruses. They have single-stranded, linear, nonsegmented, positive-polarity RNA within a naked icosahedral capsid. The name "picorna" is derived from pico (small), RNA-containing. There are two groups of human pathogens: (1) enteroviruses such as poliovirus, coxsackievirus, echovirus, and hepatitis A virus; and (2) rhinoviruses.

Caliciviruses

These are naked viruses (38 nm in diameter) with an icosahedral capsid. They have single-stranded, linear, nonsegmented, positive-polarity RNA. There are two human pathogens: Norwalk virus and hepatitis E virus.

Reoviruses

These are naked viruses (75 nm in diameter) with two icosahedral capsid coats. They have 10 segments of double-stranded linear RNA. The name is an .acronym of respiratory enteric orphan, because they were originally found in the respiratory and enteric tracts and were not associated with any human disease. The main human pathogen is rotavirus, which causes diarrhea, mainly in infants.

Flaviviruses

These are enveloped viruses with an icosahedral capsid and single-stranded, linear, nonsegmented, positive-polarity RNA. The flaviviruses include hepatitis C virus, yellow fever virus, dengue virus, and St. Louis and Japanese encephalitis viruses.

Togaviruses

These are enveloped viruses with an icosahedral capsid and single-stranded, linear, nonsegmented, positive-polarity RNA. There are two major groups of human pathogens: the alphaviruses and rubiviruses. The alphavirus group includes eastern and western encephalitis viruses; the rubivirus group consists only of rubella virus.

Retroviruses

These are enveloped viruses with an icosahedral capsid and two identical strands of single-stranded, linear, positive-polarity RNA. The term "retro" pertains to the reverse transcription of the RNA genome into DNA. There are two medically important groups: (1) the oncovirus group, which contains the sarcoma and leukemia viruses, eg, human T cell leukemia virus (HTLV); and (2) the lentivirus ("slow virus") group, which includes human immunodeficiency virus (HIV) and certain animal pathogens, eg, visna virus. A third group, spumaviruses.

Table 20. Classification of RNA viruses.

Virus Family	Envelope Present	Capsid Symmetry	Particle Size (nm)	RNA MW (x 10^6)	RNA Structure[1]	Medically Important Viruses
Picornavirus	No	Icosahedral	28	2.5	SS linear, Nonsegmented; positive Polarity	Poliovirus, rhinovirus, hepatitis A virus
Calicivirus	No	Icosahedral	38	2.7	SS linear, Nonsegmented, positive Polarity	Norwalk virus, hepatitis E virus
Reovirus	No	Icosahedral	75	15	DS linear, 10	Reovirus, rotavirus
Flavivirus	Yes	Icosahedral	45	4	SS linear, Nonsegmented, positive polarity	Yellow fever virus, dengue, virus, hepatitis C virus
Togavirus	Yes	Icosahedral	60	4	SS linear; Nonsegmented, positive Polarity	Rubella virus
Retrovirus	Yes	Icosahedral	100	7[2]	SS linear, 2 segments, positive polarity	HIV, human T-cell leukemia virus
Orthomyxovi	Yes	Helical	80-120	4	SS linear, 8 segments, negative polarity	Influenza virus
Paramyxoviru	Yes	Helical	150	6	SS linear, Nonsegmented, negative polarity	Measles virus, mumps virus, respiratory syncytial virus
Rhabdovirus	Yes	Helical	75 x 180	4	SS linear, Nonsegmented, negative polarity	Rabies virus
Filovirus	Yes	Helical	80[3]	4	SS linear, Nonsegmented; negative polarity	Ebola virus, Marburg virus
Coronavirus	Yes	Helical	100	5	SS linear, Nonseg-mented, positive Polarity	Coronavirus
Arenavirus	Yes	Helical	80-130	5	SS circular, 2 segments with cohesive ends, negative polarity	Lymphocytic choriomeningitis virus
Bunyavirus	Yes	Helical	100	5	SS circular, 3 segments with cohesive ends, negative polarity	California encephalitis virus, hanta virus
Deltavirus	Yes	Helical	37	0.5	SS circular, closed circle, negative Polarity	Hepatitis delta virus

[1] SS, single-stranded; DS, double-stranded.

[2] Retrovirus RNA contains 2 identical molecules of MW 3.5 x 10^6.

[3] Particles are 80 nm wide but can be thousands of nanometers long.

Orthomyxoviruses

These viruses (myxoviruses) are enveloped, with a helical nucleocapsid and eight segments of linear, single-stranded, negative-polarity RNA. The term "myxo" refers to the affinity of these viruses for mucins, and "ortho" is added to distinguish -them from the-paramyxoviruses. Influenza virus is the main human pathogen.

Paramyxoviruses

These are enveloped viruses with a helical nucleocapsid and singlestranded, linear, nonsegmented, negative-polarity RNA. The important human pathogens are measles, mumps, parainfluenza, and respiratory syncytial viruses.

Rhabdoviruses

These are bullet-shaped enveloped viruses with a helical nucleocapsid and a single-stranded, linear, nonsegmented, negative-polarity RNA. The term "rhabdo" refers to the bullet shape. Rabies virus is the only important human pathogen.

Filoviruses

These are enveloped viruses with a helical nucleocapsid and single-stranded, linear, nonsegmented, negative-polarity RNA. They are highly pleomorphic, long filaments that are 80 nm in diameter but can be thousands of nanometers long. The term "filo" means "thread" and refers to the long filaments. The two human pathogens are Ebola virus and Marburg virus.

Coronaviruses

These are enveloped viruses with a helical nucleocapsid and a single-stranded, linear, nonsegmented, positive-polarity RNA. The term "corona" refers to the prominent halo of spikes protruding from the envelope. Coronaviruses cause respiratory tract infections (eg, the common cold) in humans.

Arenaviruses

These are enveloped, viruses with a helical nucleocapsid and a single-stranded, circular, negative-polarity RNA in two segments. The term "arena" means "sand" and refers to granules on the virion surface that are nonfunctional ribosomes. Two human pathogens are lymphocytic choriomeningitis virus and Lassa fever virus.

Bunyaviruses

These are enveloped viruses with a helical nucleocapsid and a single-stranded, circular, negative-polarity RNA in three segments. The term "bunya" refers to the prototype, Bunyamwera virus, which is named for the place in Africa where it was isolated. These viruses cause encephalitis and various fevers such as Korean hemorrhagic fever. Hantaviruses, such as Sin Nombre virus, are members of this family.

Deltavirus

Hepatitis delta virus (HDV) is the only member of this genus. It is an enveloped virus with a helical nucleocapsid and an RNA genome that is a single-stranded, negative-polarity, covalently closed circle. It is a defective virus because it cannot replicate unless hepatitis B virus (HBV) is present within the same cell. HBV is required because it encodes hepatitis B surface antigen (HBsAg), which serves as the outer protein coat of HDV. The RNA genome of HDV encodes only one protein, the internal core protein called delta antigen.

Pathogenesis

The ability of viruses to cause disease can be viewed on two distinct levels: (1) the changes that occur within individual cells and (2) the process that takes place in the infected patient.

THE INFECTED CELL

There are four main effects of virus infection on the cell: (1) death, (2) fusion of cells to form multinucleated cells, (3) malignant transformation, and (4) no apparent morphologic or functional change. Death of the cell is probably due to inhibition4of macromolecular synthesis. Inhibition of host cell protein synthesis frequently occurs first and is probably the most important: Inhibition of DNA and RNA synthesis may be a secondary effect. It is important to note that synthesis of cellular proteins is inhibited but viral protein synthesis still occurs. For example, poliovirus inactivates an initiation factor (IF) required for cellular mRNA to be translated into cellular proteins; but poliovirus mRNA has a special ribosome-initiating site that allows it to bypass the IF so that viral proteins can be synthesized.

Infected cells frequently contain inclusion bodies, which are discrete areas containing viral proteins or viral particles. They have a characteristic intranuclear or intracytoplasmic location and appearance depending on the virus. One of the best examples of inclusion bodies that can assist in clinical diagnosis is that of Negri bodies, which are eosinophilic cytoplasmic inclusions found in rabies virus-infected brain neurons. Electron micrographs of inclusion bodies can also aid in the diagnosis when virus particles of typical morphology are visualized.

Fusion of virus-infected cells produces multinucleated giant cells, which characteristically form after infection with herpesviruses and paramyxoviruses. Fusion occurs as a result of cell membrane changes, which are probably due to the insertion of viral proteins into the membrane. The clinical diagnosis of herpesvirus skin infections is aided by the finding of multinucleated giant cells with eosinophilic intranuclear inclusions in skin scrapings.

A hallmark of viral infection of the cell is the cytopathic effect (CPE). This change in the appearance of the infected cell usually begins with a rounding and darkening of the cell and culminates in either lysis (disintegration) or giant cell formation. Detection of virus in a clinical specimen frequently is based on the appearance of CPE in cell culture. In addition, CPE is the basis for the plaque assay, an important method for quantifying the amount of virus in a sample.

Infection with certain viruses causes malignant transformation, which is characterized by unrestrained growth, prolonged survival, and morphologic changes such as focal areas of rounded, piled-up cells. Infection of the cell accompanied by virus production can occur without morphologic or gross functional changes. This observation highlights the wide variations in the nature of the interaction between the virus and the cell, ranging from rapid destruction of the cell to a symbiotic relationship in which the cell survives and multiplies despite the replication of the virus.

THE INFECTED PATIENT

Pathogenesis in the infected patient involves (1) transmission of the virus and its entry into the host; (2) replication of the virus and damage to cells; (3) spread of the virus to other cells and organs; (4) the immune response, both as a host defense and as a contributing cause of certain diseases; and (5) persistence of the virus in some instances.

The stages of a typical viral infection are the same as those described for a bacterial infection, namely, an incubation period during which the patient is asymptomatic, a prodromal period during which nonspecific symptoms occur, a specific-illness period during which the characteristic symptoms and signs occur, and a recovery period during which the illness wanes and the patient regains good health. In some patients, the infection persists and a chronic carrier state or a latent infection occurs (see below).

Transmission & Portal of Entry

Viruses are transmitted to the individual by many different routes, and their portals of entry are varied. For example, person-to-person spread occurs by transfer of respiratory secretions, saliva, blood, or semen and, by fecal contamination of water or food. Transmission can occur also between mother and offspring in utero across the placenta, at the time of delivery, or during breast feeding. Animal-to-human transmission can take place either directly from a bite of a-reservoir host as in rabies or indirectly through the bite of an insect vector, such as a mosquito, which transfers the virus from an animal reservoir to the person. In addition, activation of a latent, nonreplicating virus to form an active, replicating virus can occur within the individual, with no transmission from an external source.

Localized or Disseminated Infections

Viral infections are either localized to the portal of entry or spread systemically through the body. The best example of the localized infection is the common cold, which involves only the upper respiratory tract. Influenza is localized primarily to the upper and lower respiratory tracts. One of the best-understood systemic viral infections is paralytic poliomyelitis. After poliovirus is ingested; it infects and multiplies within the cells of the small intestine and then spreads to the mesenteric lymph nodes, where it multiplies again. It then enters the bloodstream and is transmitted to certain internal organs, where it multiplies again. The virus reenters the bloodstream and is transmitted to the central nervous system, where damage to the anterior horn cells occurs, resulting in the characteristic muscle paralysis. It is during this obligatory viremia that circulating IgG antibodies induced by the polio vaccine can prevent the virus from infecting the central nervous system. Viral replication in the gastrointestinal tract results in the presence of poliovirus in the feces, thus perpetuating its transmission to others.

Some of the molecular determinants of pathogenesis have been determined by using reovirus infection in mice as a model system. This virus has three different outer capsid proteins, each of which has a distinct function in determining the course

of the infection. One of the proteins binds to specific receptors on the cell surface and thereby determines tissue tropism. A second protein conveys resistance to proteolytic enzymes in the gastrointestinal tract and acts as the antigen that stimulates the cellular immune response. The third protein inhibits cellular RNA and protein synthesis, leading to death of the cell. Alternatively, the third protein can play a role in the initiation of persisitent viral infection.

Table 21. Main portal of entry of important viral pathogens.

Portal of Entry	Virus	Disease
Respiratory tract[1]	Influenza virus	Influenza
	Rhinovirus	Common cold
	Respiratory syncytial virus	Bronchiolitis
	Epstein-Barr virus	Infectious mononucleosis
	Varicella-zoster virus	Chickenpox
	Herpes simplex virus type 1	Herpes labialis
	Cytomegalovirus	Mononucleosis syndrome
	Measles virus	Measles
	Mumps virus	Mumps
	Rubella virus	Rubella
	Hantavirus	Pneumonia
	Adenovirus	Pneumonia
Gastrointestinal	Hepatitis A virus	Hepatitis A
	Poliovirus	Poliomvelitis
	Rotavirus	Diarrhea
Skin	Rabies virus[3]	Rabies
	Yellow fever virus[3]	Yellow fever
	Dengue virus[3]	Dengue
	Human papillomavirus	Papillomas (warts)
Genital tract	Human papillomavirus	Papillomas (warts)
	Hepatitis B virus Human	Hepatitis B
	immunodeficiency virus	AIDS
	Herpes simplex virus type 2	Herpes genitalis and neonatal herpes
Blood	Hepatits B virus Hepatits C virus Human immunodeficiency virus Cytomegalovirus	Hepatitis B Hepatitis C - AIDS, Mononucleosis syndrome or pneumonia
Transplacental	Cytomegalovirus	Congenital abnormalities
	Rubella	Congenital abnormalities

' Transmission of these viruses is typically by respiratory aerosols or saliva.

[2] Transmission of these viruses is typically by the fecal-oral route in contaminated food or water.

[3] Transmission of these viruses is typically by the bite of an infected animal.

Table 22. Viruses that cause important zoonotic diseases.

Virus	Animal Reservoir	Mode of Transmission	Disease
Rabies virus	In United States, skunks, and bats; in developing dogs.	Usually, bite of infected Also, aerosol of bat saliva.	Rabies
Hantavirus[1]	Deer mice.	Aerosol of dried excreta.	Hantavirus nary syndrome (pneumonia)
Yellow fever	Monkeys.	Bite of *Aedes* mosquito.	Yellow fever
Dengue virus	Monkeys.	Bite of *Aedes* mosquito.	Dengue
Encephalitis viruses[2]	Wild birds, eg, sparrows.	Bite of various mosquitoes.	Encephalitis

' Sin Nombre virus is the most important hantavirus in he United States.

[2] Important encephalitis viruses in the United States include eastern and western equine encephalitis viruses and St. Louis encephalitis virus.

However, there are certain diseases in which cell killing by immunologic attack plays an important role in pathogenesis. Both cytotoxic T cells and antibodies play a role in immunopathogenesis.

(1) The best-studied system is lymphocytic choriomeningitis (LCM) in mice; LCM occurs in humans also but is quite rare. When LCM virus is inoculated into the brain of an adult mouse, virus replication occurs and death follows. However, when LCM virus is inoculated into the brain of an immunosuppressed adult mouse or a newborn mouse, the animal remains well despite extensive virus replication. When immune lymphocytes are inoculated into these infected, healthy mice, death ensues. It appears that death of the cells is caused by immune attack by cytotoxic T cells on the new viral antigens in the cell membrane rather than by virus-induced inhibition of cell functions.

(2) Cytotoxic T cells are involved in the pathogenesis of hepatitis caused by hepatitis A, B, and C viruses. These viruses do not cause a cytopathic effect, and the damage to the hepatocytes is the result of the recognition of viral antigens on the hepatocyte surface by cytotoxic T cells. The rash of measles is similarly caused by these cells attacking the infected vascular endothelium in the skin.

(3) Immune-mediated pathogenesis also occurs when virus-antibody-complement complexes form and are deposited in various tissues. This occurs in hepatitis B virus infection, in which immune complexes play a role in producing the arthritis characteristic of the early stage of hepatitis B. The pathogenesis of pneumonia caused by respiratory syncytial virus in infants is attributed to immune complexes formed by maternal IgG and viral antigens.

Virulence

Strains of viruses differ greatly in their ability to cause disease. For example, there are strains of poliovirus which have mutated sufficiently that they have lost the

ability to cause polio in immunocompetent individuals; ie, they are attenuated. These strains are used in vaccines. The viral genes that control the virulence of the virus are poorly characterized, and the process of virulence is poorly understood.

In the early 1990s, some insight was gained with the finding that some viruses encode the receptors for various mediators of immunity such as interleukin-1 (IL-1) and tumor necrosis factor (TNF). For example, vaccinia virus encodes a protein that binds to IL-1 and fibroma virus encodes a protein that binds to TNF. When released from virus-infected cells, these proteins bind to the immune mediators and block their ability to interact with receptors on their intended targets, our immune cells that mediate host defenses against the viral infection. By reducing our host defenses, the virulence of the virus is enhanced. In addition, some viruses (eg, HIV) can reduce the expression of class I MHC proteins, thereby reducing the ability of cytotoxic T cells to kill the virus-infected cells, and others (eg, herpes simplex virus) inhibit complement. Several viruses (HIV, Epstein-Barr virus, and adenovirus) synthesize RNAs that block the phosphorylation of an initiation factor (eIF-2), which reduces the ability of interferon to block viral replication. Collectively, these viral virulence factors are called virokines.

Persistent Viral Infections

In most viral infections, the virus does not remain in the body for a significant period after clinical recovery. However, in certain instances, the virus persists for long periods either intact or in the form of a subviral component, eg, the genome. The mechanisms that may play a role in the persistence of viruses include (1) integration of a DNA provirus into host cell DNA, as occurs with retroviruses; (2) immune tolerance, because neutralizing antibodies are not formed; (3) formation of virus-antibody complexes, which remain infectious; (4) location within an immunologically sheltered "sanctuary," eg, the brain; (5) rapid antigenic variation; (6) spread from cell to cell without an extracellular phase, so that virus is not exposed to antibody; and (7) immunosuppression, as in AIDS.

There are three types of persistent viral infections of clinical importance. They are distinguished primarily by whether virus is usually produced by the infected cells and by the timing of the appearance both of the virus and of the symptoms of. disease.

A. Chronic-Carrier Infections:

Some patients who have been infected with certain viruses continue to produce significant amounts of the virus for long periods. This carrier state can follow an asymptomatic infection as well as the actual disease and can itself either be asymptomatic or result in chronic illness. Important clinical examples are chronic hepatitis, which occurs in hepatitis B and hepatitis C virus carriers, and neonatal rubella virus and cytomegalovirus infections, in which carriers can produce virus for years.

B. Latent Infections:

In these infections, best illustrated by the herpesvirus group, the patient recovers from the initial infection and virus production stops. Subsequently, the symptoms may recur, accompanied by the production of virus. In herpes simplex virus infections, the virus enters the latent state in the cells of the sensory ganglia.

`The molecular nature of the latent state is unknown. Herpes simplex virus type 1, which causes infections primarily of the eyes and face, is latent in the trigeminal ganglion, whereas herpes simplex virus type 2, which causes infections primarily of the genitals, is latent in the- lumbar and sacral ganglia. Varicella-zoster virus, another member of the herpesvirus family, causes varicella (chickenpox) as its initial manifestation and then remains latent, primarily in the trigeminal or thoracic ganglion cells. It can

C. Slow Virus Infections:

The term "slow virus" refers to the prolonged period between the initial infection and the onset of disease, which is usually measured in years. In instances in which the cause has been identified, the virus has been shown to have a normal, not prolonged, growth cycle. It is not, therefore, that virus growth is slow; rather, the incubation period and the progression of the disease are prolonged. Two of these diseases are caused by conventional viruses, namely, subacute sclerosing panencephalitis, which follows several years after measles virus infections, and progressive multifocal leukoencephalopathy (PML), which is caused by JC virus, a papovavirus. PML occurs primarily in patients who have lymphomas or are immunosuppressed. Other slow infections in humans, eg, Creutzfeldt-Jakob disease and kuru, may be caused by unconventional agents called prions. Slow virus infections are described later.

Host Defenses

Host defenses against viruses fall into two major categories: (1) nonspecific, of which the most important are interferons; and (2) specific, including both humoral and cell-mediated immunity. Interferons are an early, first-line defense, whereas humoral immunity and cell-mediated immunity are effective only later since it takes several days to induce an immune response.

NONSPECIFIC DEFENSES

1. Interferons

Interferons are a heterogeneous group of glycoproteins produced by human and other animal cells after viral infection (or after exposure to other inducers). They inhibit the growth of viruses by blocking the translation of viral proteins. Interferons are divided into three groups based on the cell of origin, namely, leukocyte, fibroblast, and lymphocyte. They are also known as alpha, beta, and gamma interferons, respectively. Alpha and beta interferons are induced by viruses, whereas gamma (T cell, immune) interferon is induced by antigens and is one of the effectors of cell-mediated immunity. Interferons are cytokines that inhibit the growth of certain cancer cells, bacteria, and protozoa, but the focus here will be on their inhibitory effect on viral growth. The following discussion of alpha and beta interferons focuses on two aspects of their antiviral effect: induction and action.

Induction of Alpha & Beta Interferons

The strong inducers of these interferons are viruses and double-stranded RNAs. Induction is not specific for a particular virus; many DNA and RNA viruses of humans, other animals, plants, and bacteria are competent, although they differ in

effectiveness. The finding that double-stranded RNA, but not single-stranded RNA or DNA, is a good inducer has led to the hypothesis that a double-stranded RNA is synthesized as part of the replicative cycle of all inducing viruses. The double-stranded RNA poly(rI-rC) is one of the strongest inducers and was under consideration as an antiviral agent, but toxic side effects prevented its clinical use. The weak inducers of microbiologic interest include a variety of intracellular bacteria and protozoa, as well as certain bacterial substances such as endotoxin.

This extensive list of inducers makes it clear that induction of these interferons is not specific. Similarly, their inhibitory action is not specific for any particular virus. However. they are typically specific in regard to the host species in which they act; ie, interferons produced by human cells are active in human cells but are much less effective in cells of other species. It is clear, therefore, that other animals cannot be used as a source of interferons for human therapy. Rather. the genes for human interferons have been cloned and material for medical trials is now produced by genetic engineering techniques.

Action of Alpha & Beta Interferons

Interferons inhibit the intracellular replication of a wide variety of viruses but have little effect on the metabolism of normal cells; ie. they exhibit a remarkable degree of selective inhibition. They act by inducing the synthesis of three proteins that inhibit the translation of viral mRNA without affecting the translation of cellular mRNA. The three proteins are (1) a 2,5-oligonucleotide synthetase that synthesizes an adenine trinucleotide [2,5oligo(A)]; (2) a ribonuclease that is activated by 2,5-oligo(A) and that degrades viral but not cellular mRNAs; and (3) a protein kinase that phosphorylates an initiation factor for protein synthesis (eIF-2), thereby inactivating it. The endonuclease selectively degrades viral mRNAs by recognizing a nucleotide sequence on viral mRNAs that is not present on cellular mRNAs. Interferons have no direct effect on extracellular virus particles.

Because interferons are produced within a few hours of the initiation of viral replication, they may act in the early phase of viral diseases to limit the spread of virus. In contrast, antibody begins to appear in the blood several days after infection.

Alpha interferon has been approved for use in patients with condyloma acuminatum and chronic active hepatitis caused by hepatitis C virus. Gamma interferon reduces recurrent infections in patients with chronic granulomatous disease. Interferons are also used clinically in patients with cancers such as Kaposi's sarcoma and hairy cell leukemia.

2. Phagocytosis

Macrophages, particularly fixed macrophages of the reticuloendothelial system and alveolar macrophages, are the important cell types in limiting virus infection. In contrast, polymorphonuclear leukocytes are the predominant cellular defense in bacterial infections.

3. Fever

Elevated body temperature may play a role in host defenses, but its importance is uncertain. Fever may act in two ways. (1) The higher body temperature may directly inactivate the virus particles, particularly enveloped viruses, which are

more heat-sensitive than nonenveloped viruses. (2) Replication of some viruses is reduced at higher temperatures, and so fever may inhibit replication.

4. Mucociliary Clearance

The mucociliary clearance mechanism of the respiratory tract may protect the host. Its damage, eg, from smoking, results in an increased frequency of viral respiratory tract infections, especially influenza.

5. Factors that Modify Host Defenses

Several factors influence host defenses in a nonspecific or multifactorial way.

(1) Age is a significant variable in the outcome of viral infections. In general, infections are more severe in neonates and in the elderly than in older children and young adults. For example, influenza is typically more severe in older people than in younger adults and herpes simplex virus infections are more severe in neonates than in adults.

(2) Increased corticosteroid levels predispose to more severe infections with some viruses, such as varicella-zoster virus; the use of topical cortisone in herpetic keratitis can exacerbate eye damage. It is not clear how these effects are mediated, because corticosteroids can cause a variety of pertinent effects, namely, lysis of lymphocytes, decreased recruitment of monocytes, inhibition of interferon production, and stabilization of lysosomes.

(3) Malnutrition leads to more severe viral infections; eg, there is a much higher death rate from measles in developing countries than in developed ones. Poor nutrition causes decreased immunoglobulin production and phagocyte activity as well as reduced skin and mucous membrane integrity.

SPECIFIC DEFENSES

There is evidence for natural resistance to some viruses in certain species, which is probably based on the absence of receptors on the cells of the resistant species. However, by far the most important type of defense is acquired immunity, either actively acquired by exposure to the virus or passively acquired by the transfer of immune serum. Active immunity can be elicited by contracting the actual disease, by having an inapparent infection, or by being vaccinated.

1. Active Immunity

Active immunity is important in the prevention of disease, but its ability to enhance the patient's recovery from a viral disease is limited. Disease prevention is chiefly due to the presence of immunoglobulins; eg, passive transfer of serum containing specific antibodies is typically protective. Cell-mediated -immunity is also important, because when it is suppressed, severe viral infections commonly occur, eg, in AIDS patients.

The duration of protection varies; disseminated viral infections such as measles and mumps confer lifelong immunity against recurrences, but localized infections such as the common cold usually impart only a brief immunity of several` months. IgA confers protection against viruses that enter through the respiratory and gastrointestinal mucosa, and IgM and IgG protect against viruses that enter or are

spread through the blood. The lifelong protection against systemic viral infections such as the childhood diseases measles, mumps, rubella, and chickenpox (varicella) is a function of the anamnestic (secondary) response of IgG. For certain respiratory viruses such as parainfluenza and respiratory syncytial viruses, the IgA titer in respiratory secretions correlates with protection, whereas the IgG titer does not. Unfortunately, protection by IgA against most respiratory tract viruses usually lasts less than 5 years.

The role of active immunity in recovery from a viral infection is uncertain. Because recovery usually precedes the appearance of detectable humoral antibody, immunoglobulins may not be important. Also, children with agammaglobulinemia recover from measles infections normally and can be immunized against measles successfully, indicating that cell-mediated immunity plays an important role. This is supported by the observation that children with congenital T cell deficiency are vulnerable to severe infections with measles virus and herpesviruses. T cells are important in recovery from many but not all viral illnesses.

The protection offered by active immunity can be affected by the phenomenon of "original antigenic sin." This term refers to the observation that when a person is exposed to a virus that crossreacts with another virus to which that individual was previously exposed, more antibody may be produced against the original virus than against the current one. It appears that the immunologic memory cells can respond to the original antigenic exposure to a greater extent than to the subsequent one. This was observed in people with antibodies to the A_I type of influenza virus, who, when exposed to the A_2 type, produced large amounts of antibody to A_1 but very little antibody to the A_2 virus. It is also the underlying cause of severe hemorrhagic dengue fever. This phenomenon has two practical consequences as well: (1) Attempts to vaccinate people against the different influenza virus strains may, be less effective than expected, and (2) epidemiologic studies based on measurement of antibody titers may yield misleading results.

How does antibody inhibit viruses? There are two main mechanisms. The first is neutralization of the infectivity of the virus by antibody binding to the proteins on the outer surface of the virus. This binding has two effects: (1) It can prevent the interaction of the virus with cell receptors, and (2) it can cross-link the viral proteins and stabilize the virus so that uncoating does not occur. The virus therefore cannot replicate. Furthermore, antibody-coated virus is more rapidly phagocytized than normal virus, a process similar to the opsonizing effect of antibody on bacteria. Antibody does not degrade the virus particle; fully infectious virus can be recovered by dissociating the virus-antibody complex. Incomplete or "blocking" antibody can interfere with neutralization and form immune complexes, which are important in the pathogenesis of certain diseases. Some viruses, such as herpesviruses, can spread from cell to cell across intercellular bridges, eluding the neutralizing effect of antibody.

The second mechanism is the lysis of virus-infected cells in the presence of antibody and complement. Antibody binds to new virus-specific antigens on the cell

surface and then binds complement, which enzymatically degrades the cell membrane. Because the cell is killed before the full yield of virus is produced, the spread of virus is significantly reduced.

Lysis of virus-infected cells is also caused by cytotoxic T lymphocytes. These CD-8 positive T cells recognize viral antigen only when it is presented in association with class I MHC proteins. They kill virus-infected cells by three methods: (1) by releasing perforins, which make holes in the cell membrane of the infected cells, (2) by releasing enzymes called granzymes into the infected cell which degrade the cell contents, and (3) by activating the FAS protein, which causes programmed cell death (apoptosis).

Not all virus infections induce antibodies. Tolerance to viral antigens can occur when the virus infection develops in a fetus or newborn infant. The model system in which tolerance has been demonstrated is lymphocytic choriomeningitis (LCM) infection in mice. If LCM virus is inoculated into a newborn mouse, the virus replicates widely but no antibodies are formed during the lifetime of the animal. The virus is recognized as "self," because it was present at the time of maturation of the immune system. If LCM virus is given to an adult mouse, antibodies are formed normally. There is no example of total tolerance to a virus in humans; even in congenital rubella syndrome, in which the virus infects the fetus, some antibody against rubella virus is made. However, virus production and shedding can go on for months or years.

Suppression of the cell-mediated response can occur during infection by certain viruses. The bestknown example is the loss of tuberculin skin test reactivity during measles infection. Infection by cytomegalovirus or human immunodeficiency virus can also cause suppression. Some viruses can "down-regulate" (reduce) the amount of class I and class II MHC protein made by cells, which may be a mechanism by which these viruses suppress cell-mediated immunity.

2. Passive Immunity

Transfer of human serum containing the appropriate antibodies provides prompt short-term immunity for individuals exposed to certain viruses. The term "passive" refers to the administration of preformed antibodies. Two types of immune globulin preparations are used for this purpose. One has a high titer of antibody against a specific virus, and the other is a pooled sample from plasma donors that contains a heterogeneous mixture of antibodies with lower titers. The immune globulins are prepared by alcohol fractionation, which removes any viruses in the serum. The three most frequently used high-titer preparations are used after exposure to hepatitis B, rabies, and varicellazoster viruses. Low-titer immune globulin is used mainly to prevent hepatitis A in people traveling to areas where this infection is hyperendemic.

Laboratory Diagnosis

There are five approaches to the diagnosis of viral diseases by the use of clinical specimens: (1) identification of the virus in cell culture, (2) microscopic identification directly in the specimen, (3) serologic procedures to detect a rise in antibody titer or the presence of IgM antibody, (4) detection of viral antigens in blood or body fluids, and (5) detection of viral nucleic acids in blood or the patient's cells.

IDENTIFICATION IN CELL CULTURE

The growth of viruses requires cell cultures, because viruses replicate only in living cells, not on cell-free media the way most bacteria can. Because many viruses are inactivated at room temperature, it is important to inoculate the specimen into the cell culture as soon as possible; brief transport or storage at 4 °C is acceptable. Virus growth in cell culture frequently produces a characteristic cytopathic effect (CPE) that can provide a presumptive identification. The time taken for the CPE to appear and the type of cell in which the virus produces the CPE are important clues in the presumptive identification.

If the virus does not produce a CPE, its presence can be detected by several other techniques:

(1) Hemadsorption, ie, attachment of erythrocytes to the surface of virus-infected cells. This technique is limited to viruses with a hemagglutin n prot6in on their envelope, such as mumps, parainfluenza, and influenza viruses.

(2) Interference with the formation of a CPE by a second virus. For example, rubella virus, which does not cause a CPE, can be detected by interference with the formation of. a CPE by certain enteroviruses such as echovirus or coxsackievirus.

(3) A decrease in acid production by infected, dying cells. This can be detected visually by the color change in the phenol red (a pH indicator) in the culture medium. The indicator remains red (alkaline) in the presence of virus-infected cells but turns yellow in the presence of metabolizing normal cells as a result of the acid produced. This can be used to detect certain enteroviruses.

A definitive identification of the virus grown in cell culture is made by using known antibody in one of several tests. Complement fixation, hemagglutination inhibition, and neutralization of the CPE are the most frequently used tests. Other procedures such as fluorescent antibody, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), and immunoelectron microscopy are also used in special instances. A brief outline of these tests follows. They are described in more detail in the section on immunology.

Complement Fixation If the antigen (the unknown virus in the culture fluid) and the known antibody are homologous, complement will be fixed (bound) to the antigen-antibody complex. This makes it unavailable to lyse the "indicator" system, which is composed of sensitized red blood cells.

Hemagglutination Inhibition

If the virus and antibody are homologous, the virus is blocked from attaching to the erythrocytes and no hemagglutination occurs. Only viruses that agglutinate red blood cells can be identified by this method.

Neutralization

If the virus and antibody are homologous, the antibody bound to the surface of the virus blocks its entry into the cell. This neutralizes viral infectivity, because it prevents viral replication and subsequent CPE formation or animal infection.

Fluorescent-Antibody Assay

If the virus-infected cells and the fluorescein-tagged antibody are homologous, then the typical apple-green color of fluorescein is seen in the cells by ultraviolet (UV) microscopy.

Radioimmunoassay

If the virus and the antibody are homologous, there is less antibody remaining to bind to the known radiolabeled virus.

Enzyme-Linked Immunosorbent Assay (ELISA)

First, the antibody is bound to a surface. If the virus is homologous, it will be bound also. A sample of the antibody linked to an enzyme is added, and the amount of enzyme is assayed.

Immunoelectron Microscopy

If the antibody is homologous to the virus, aggregates of virus-antibody complexes are seen in the electron microscope.

MICROSCOPIC IDENTIFICATION

Viruses can be detected and identified by direct microscopic examination of clinical specimens such as biopsy material or skin lesions. Three different procedures can be used. (1) Light microscopy can reveal characteristic inclusion bodies or multinucleated giant cells. The Tzanck-smear, which shows herpesvirus-induced multinucleated giant cells in vesicular skin lesions, is a good example. (2) UV microscopy is used for fluorescent-antibody staining of the virus in infected cells. (3) Electron microscopy detects virus particles, which can be characterized by their size and morphology.

SEROLOGIC PROCEDURES

In the third approach, a rise in the titer (Titer is a measure of the concentration of antibodies in the patient's serum. It is defined as the highest dilution of serum that gives a positive reaction in the test) of antibody to the virus can be used to indicate current infection. A serum sample is obtained as soon as a viral etiology is suspected (acute-phase), and a second sample is obtained 10-14 days later (convalescent-phase). If the antibody titer in the convalescent phase serum sample is at least 4-fold higher than the titer in the acute-phase serum sample, the patient is considered to be infected. For example, if the titer in the acute-phase serum sample is 1/4 and the titer in the convalescent-phase serum sample is 1/16 or greater, the patient has had a significant rise in antibody titer and has been recently infected. If, however, the titer in the convalescent-phase serum sample is 1/8, this is not a significant rise and should not be interpreted as a sign of recent infection.

It is important to realize that an antibody titer on a single sample does not distinguish between a previous infection and a current one. The antibody titer can be determined by many of the immunologic tests mentioned above. These serologic diagnoses are usually made retrospectively, because the disease has frequently run its course by the time the results are obtained.

In certain viral diseases, the presence of IgM antibody is used to diagnose current infection. For example, the presence of IgM antibody to core antigen indicates infection by hepatitis B virus. Other nonspecific serologic tests are available. For example, the heterophil antibody test (Monospot) can be used to diagnose infectious mononucleosis.

DETECTION OF VIRAL ANTIGENS

Viral antigens can be detected in the patient's blood or body fluids by various tests but most often by an ELISA. Tests for the p24 antigen of HIV and the surface antigen of HB V are common examples of this approach.

DETECTION OF VIRAL NUCLEIC ACIDS

Viral nucleic acids, ie, either the viral genome or viral mRNA, can be detected in the patient's blood or tissues with complementary DNA or RNA (cDNA or cRNA) as a probe. If only small amounts of viral nucleic acids are present in the patient, the polymerase chain reaction (PCR) can be used to amplify the viral nucleic acids. Assays for the RNA of HIV in the patient's blood (viral load) are commonly used to monitor the course of the disease and to evaluate the patient's prognosis

HEPATITIS VIRUSES

Many viruses cause hepatitis. Of these, five medically important viruses are commonly described as "hepatitis viruses": hepatitis A virus (HAV); hepatitis B virus (HBV); non-A, non-B viruses, of which hepatitis C virus (HCV) is the most common; hepatitis D virus (HDV, delta agent); and hepatitis E virus (HEV) (Tables 23 and 24). Other viruses, such as Epstein-Barr virus (the cause of infectious mononucleosis), cytomegalovirus, and yellow fever virus, cause inflammation of the liver but are not called hepatitis viruses per se. They are discussed elsewhere.

Table 23. Glossary of hepatitis viruses and their serologic markers.

Abbreviation	Name and Description
HAV	Hepatitis A virus (enterovirus 72), a picornavirus (nonenveloped RNA
IgM HAVAb	IgM antibody to HAV; best test to detect acute hepatitis A.
HBV	Hepatitis B virus, a hepadnavirus (enveloped, partially double-stranded DNA virus); also
HBsAg	Antigen found on surface of HBV, also found on noninfectious particles in patient's blood;
HBsAb	Antibody to HBsAg; provides immunity to hepatitis B.
HBcAg	Antigen associated with core of HBV.
HBcAb	Antibody to HBcAg; positive during window phase. IgM HBcAb is an indicator of recent
HBsAg	A second, different antigenic determinant in the HBV cores Important
HBeAb	Antibody to e antigen; indicates low transmissibility.
Non-A, non-B	Hepatitis viruses that are neither HAV nor HBV.
HCV	Enveloped RNA virus; one of the non-A, non-B viruses:
HEV	Nonenveloped RNA virus; one of the non-A, non-B viruses.
HDV (delta agent)	Small RNA virus with HBsAg envelope; defective virus that replicates only in HBV-infected

HEPATITIS A VIRUS

Disease

HAV causes hepatitis A.

Important Properties

HAV is a typical enterovirus classified in the picornavirus family. It has a single-stranded RNA genome and a nonenveloped icosahedral nucleocapsid and replicates in the cytoplasm of the cell. It is also known as enterovirus 72. It has one serotype, and there is no antigenic relationship to HBV or other hepatitis viruses.

Summary of Replicative Cycle

HAV has a replicative cycle similar to that of other enteroviruses.

Transmission & Epidemiology

HAV is transmitted by the fecal-oral route. Humans are the reservoir for HAV. Virus appears in the feces roughly 2 weeks before the appearance of symptoms, so quarantine of patients is ineffective. Children are the most frequently infected group, and outbreaks occur in special living situations such as summer camps and boarding schools. Commonsource outbreaks arise from fecally contaminated water or food such as oysters grown in polluted water and eaten raw. Unlike HBV, HAV is rarely transmitted via the blood, because the level of viremia is low and chronic infection does not occur. About 50-75% of adults in the United States have been infected, as evidenced by IgG antibody.

Pathogenesis & Immunity

The pathogenesis of HAV infection is not completely understood.

The virus probably replicates in the gastrointestinal tract and spreads to the liver via the blood.

Table 24. Important properties of hepatitis viruses.

Virus	Genome	Replication Defective	DNA Polymerase in Virion	HBsAg in Envelope	Virus Family
HAV	SsRNA	No	No	No	Picornavirus
HBV	DsDNA'	No	Yes	Yes	Hepadnavirus
HCV	SsRNA	No	No	No	Flavivirus
HDV	SsRNA	Yes	No	Yes	Deitavirus
HEV	SsRNA	No	No	L No	(Calicivirus

[1] Interrupted, circular dsDNA.

[2] Circular, negative-stranded ssRNA.

Table 25. Clinical features of hepatitis viruses.

Virus	Mode of Transmission	Chronic Carriers	Laboratory Test Usually Used for Diagnosis	Vaccine Available	Immune Globulins Useful
HAV	Fecal-oral	No	IgM HAV	Yes	Yes
HBV	Blood,' sexual, at	Yes	HBsAg, HBsAb, IgM	Yes	Yes
HCV	Blood, sexual'	Yes	HCV Ab	No	No
HDV	Blood, sexual'	Yes	Ab to delta Ag	No	No
HEV	Fecal-oral	No	None	No	No

' Sexual transmission seems likely but is poorly documented.

Hepatocytes are infected, but the mechanism by which cell damage occurs is unclear. HAV infection of cultured cells produces no cytopathic effect. It is likely that attack by cytotoxic T cells causes the damage to the hepatocytes. The infection is cleared, the damage is repaired, and no chronic infection ensues. Hepatitis caused by the different viruses cannot be distinguished pathologically.

The immune response consists initially of IgM antibody, which is detectable at the time jaundice appears. It is therefore important in the laboratory diagnosis of hepatitis A. The appearance of IgM is followed 1-3 weeks later by the production of IgG antibody, which provides lifelong protection.

Clinical Findings

The clinical manifestations of hepatitis are virtually the same, regardless of which hepatitis virus is the cause (Table 41-3). Fever, anorexia, nausea, vomiting, and jaundice are typical. Dark urine, pale feces, and elevated transaminase levels are seen. Most cases resolve spontaneously in 2--4 weeks. Hepatitis A has a short incubation period (3-4 weeks), in contrast to that of hepatitis B, which is 10-12 weeks. Most HAV infections are asymptomatic and are detected solely by the presence of IgG antibody. No chronic hepatitis or chronic carrier state occurs, and there is no predisposition to hepatocellular carcinoma.

Laboratory Diagnosis

The detection of IgM antibody is the most important test. A 4-fold rise in IgG antibody titer can also be used. Isolation of the virus in cell culture is possible but not available in the clinical laboratory.

Treatment & Prevention

No antiviral therapy is available. Active immunization with a vaccine containing inactivated HAV is available. The virus is grown in human cell culture and inactivated with formalin. An initial dose followed by a booster 6 to 12 months later should be given to adults. The vaccine is recommended for travelers to developing countries. However, because many people have antibodies to HAV, it may be cost-effective to determine whether antibodies are present before giving the vaccine. Passive immunization with immune serum globulin prior to infection or early in the incubation period can prevent or mitigate the disease. Observation of proper hygiene, eg, sewage disposal and hand washing after bowel movements, is of prime importance.

HEPATITIS B VIRUS

Disease

HBV causes hepatitis B.

Important Properties

HBV is a member of the hepadnavirus family. It is a 42-nm enveloped virion,* Also known as a Dane particle (named for the scientist who first published electron micrographs of the virion). tHBsA,1 was known as Australia antigen, because it was first found in the serum of an Australian aborigine.with an icosahedral nucleocapsid core containing a partially double-stranded circular DNA genome. The envelope contains a protein called the surface antigen (HBsAg), which is important for laboratory

diagnosis and immunization] Within the core is a DNA-dependent DNA polymerase. The genome encodes only five proteins: surface antigen, core antigen, DNA polymerase, and two regulatory proteins that activate transcription of RNA.

Electron microscopy of a patient's serum reveals three different types of particles: a few 42-nm virions and many 22-nm spheres and long filaments 22 nm wide, which are composed of surface antigen. HBV is the only human virus that produces these spheres and filaments in such large numbers in the patient's blood. In addition to HBsAg, there are two other important antigens: the core antigen (HBcAg) and the e antigen (HBsAg), both of which are located in the core but have different antigenicities. HBsAg is an important indicator of transmissibility.

For vaccine purposes, HBV has one serotype based on HBsAg. However, for epidemiologic purposes, there are four serologic subtypes of HBsAg based on a group-specific antigen, "a," and two sets of mutually exclusive epitopes, d or y and w or r. This leads to four serotypes-adw, adr, ayw, and ayr-which are useful in epidemiologic studies because they are concentrated in certain geographic areas.

Humans are the only natural hosts of HBV.

Summary of Replleative Cycle

After entry of the virion into the cell and its uncoating. the virion DNA polymerase synthesizes the missing portion of DNA and a double-stranded closedcircular DNA is formed in the nucleus. This DNA serves as a template for rnRNA synthesis by cellular RNA polymerase. mRNA not only functions in protein synthesis but also is the template for the minus strand of the progeny DNA. The minus strand then serves as the template for the plus strand of the genome DNA. This RNA-dependent DNA synthesis takes place within the newly assembled virion core in the cytoplasm. Hepadnaviruses are the only viruses that produce genome DNA by reverse transcription with mRNA as the template. (Note that this type of RNA-dependent DNA synthesis is similar to but different from the process in retroviruses, in which the genome RNA is transcribed into a DNA intermediate.) Some of the progeny DNA integrates into the host cell genome, and this seems likely to be the DNA that maintains the carrier state. Progeny HBV with its HBsAg-containing envelope is released from the cell by budding through the cell membrane.

Transmission & Epidemiology

The three main modes of transmission are via blood, during sexual intercourse, and perinatally from mother to newborn. The observation that needle-stick injuries can transmit the virus indicates that only very small amounts of blood are necessary. HBV infection is especially prevalent in addicts who use intravenous drugs. Screening of blood for the presence of HBsAg has greatly decreased the number of transfusion-associated cases of hepatitis B.* *In the United States, donated blood is screened for HBsAg and antibodies to HBcAg, HCV, HIV-1; HIV-2, and HTLV-I. Two other tests are also performed: a VDRL tdst for syphilis and a transaminase assay, which, if elevated, indicates liver damage and is a surrogate marker of viral infection.

However, because blood transfusion is a modern procedure, there must be another, natural route of transmission. It is likely that sexual transmission and transmission from mother to child during birth or breast-feeding are the natural routes. Note that enveloped viruses, such as HBV, are more sensitive to the environment than nonenveloped viruses and hence are more efficiently transmitted by intimate contact, eg, sexual contact. Nonenveloped viruses, such as HAV, are quite stable and are transmitted well via the environment, eg, fecal-oral transmission.

Hepatitis B is found worldwide but is particularly prevalent in the Orient. In that region, there is a high incidence of hepatocellular carcinoma (hepatoma), a finding which indicates that HBV may be a human tumor virus. Immunization against HBV in Taiwan has significantly reduced the incidence of hepatoma in children. It appears that the HBV vaccine is the first vaccine to prevent a human cancer.

Pathogenesis & Immunity

After entering the blood, the virus infects hepatocytes, and viral antigens are displayed on the surface of the cells. Cytotoxic T cells mediate an immune attack against the viral antigens, and inflammation and necrosis occur. Immune attack against viral antigens on infected hepatocytes is mediated by cytotoxic T cells. The pathogenesis of hepatitis B is probably the result of this cell-mediated immune injury, because HBV itself does not cause a cytopathic effect. Antigen-antibody complexes cause some of the early symptoms, eg, arthralgias, and some of the complications in chronic hepatitis, eg, immune-complex glomerulonephritis and vasculitis.

Unlike hepatitis A patients, about 5% of patients with hepatitis B become chronic carriers of HBV. A chronic carrier is someone who has HBsAg persisting in their blood for at least 6 months. The chronic carrier state is attributed to a persistent infection of the hepatocytes which results in the prolonged presence of HBV and HBsAg in the blood. The main determinant of whether a person clears the infection or becomes a chronic carrier is the adequacy of the cytotoxic T cell response. HBV DNA exists primarily as an episome in the cytoplasm of persistently infected cells; a small number of copies of HBV DNA are integrated into cell DNA. A high rate of hepatocellular carcinoma occurs in chronic carriers. The HBV genome has no oncogene, and hepatocellular carcinoma appears to be the result of persistent cellular regeneration that attempts to replace the dead hepatocytes. Chronic carriage is more likely to occur when infection occurs in a newborn than in an adult, probably because a newborn's immune system is less competent than an adult's. Approximately 90% of those infected as neonates become chronic carriers.

Lifelong immunity occurs after the natural infection and is mediated by humoral antibody against HBsAg. Antibody against HBcAg is not protective.

Clinical Findings

Many HBV infections are asymptomatic and are detected only by the presence of antibody to HBsAg. The mean incubation period for hepatitis B is 10-12 weeks, which is much longer than that of hepatitis A (3-4 weeks). The clinical appearance of acute hepatitis B is similar to that of hepatitis A. However, with hepatitis B, symptoms tend to be more severe and lifethreatening hepatitis can occur. Most chronic carriers are asymptomatic, but some have chronic active hepatitis, which can lead to cirrhosis and death.

Laboratory Diagnosis

The most important laboratory test for the detection of early HBV infection is the immunoassay for HBsAg. HBsAg appears during the incubation period and is detectable in most patients during the prodrome and acute disease (Fig 41-2). It falls to undetectable levels during convalescence in most cases; its prolonged presence (at least 6 months) indicates the carrier state and the risk of chronic hepatitis and hepatic carcinoma. As described in Table 41-4, HBsAb is not detectable in the chronic carrier state. Note that HBsAb is, in fact, being made but is not detectable in the laboratory tests because it is bound to the large amount of HBsAg present in the blood. HBsAb is also being made during the acute dsease but is similarly undetectable because it is bound in immune complexes.

Note that there is a period of several weeks when HBsAg has disappeared but HBsAb is not yet detectable. This is the "window phase." At this time, the HBcAb is always positive and can be used to make the diagnosis. HBcAb is present in those with acute infection and chronic infection, as well as those who have recovered from acute infection. Therefore, it cannot be used to distinguish between acute and chronic infection. The IgM form of HBcAb is present during acute infection and disappears approximately 6 months after infection. The test for HBcAg is not readily available. Table 41-4 describes the serologic test results that characterize the four important stages of HBV infection.

HBcAg arises during the incubation period and is present during the prodrome and early acute disease, and in certain chronic carriers. Its presence is an important indicator of transmissibility, and, conversely, the finding of HBeAb indicates low transmissibility. DNA polymerase activity is detectable during the incubation period and early in the disease, but the assay is not available in most clinical laboratories. The detection of viral DNA in the serum is strong evidence that infectious virions are present.

Treatment & Prevention

Alpha interferon is clinically useful for the treatment of chronic hepatitis B infections. Some nucleoside analogues, such as lamivudine (thiacytidine), that inhibit the reverse transcriptase of: HIV; also are effective against HBV. These drugs reduce hepatic inflammation and lower the levels of HBV in chronic carriers.

Prevention involves the use of either the vaccine or hyperimmune globulin, or both.

(1) The vaccine contains HBsAg produced in yeasts by genetic engineering techniques. The vaccine is highly effective in preventing hepatitis B and has few side effects. It is indicated for people who are frequently exposed to blood or blood products, such as certain health care personnel (eg, medical students, surgeons, and dentists), patients receiving multiple transfusions or dialysis, patients with frequent sexually transmitted disease, and abusers of illicit intravenous drugs. The U.S. Public Health Service recommends that all newborns and adolescents receive the vaccine. At present, booster doses after the initial three-dose regimen are not recommended.

Table 26. Serologic test results in four stages of HBV infection.

Test	Acute Disease	Window Phase	Complete Recovery	Chronic Carrier State
HBsAg	Positive	Negative	Negative	Positive
HbsAb	Negative	Negative	Positive	Negative
HBcAb	Positive'	Positive	Positive	Positive

' IgM is found in the acute stage; IgG is found in subsequent stages.

Note: People immunized with HBV vaccine have HBsAb but not HBcAb because the immunogen in the vaccine' is purified HBsAg.

(2) Hepatitis B immune globulin (HBIG) contains a high titer of HBsAb, because it is prepared from sera of patients who have recovered from hepatitis B. It is used to provide immediate, passive protection to individuals known to be exposed to HBsAg-positive blood, eg, after an accidental needle stick.

Precise recommendations for use of the vaccine and HBIG are beyond the scope of this book. However, the recommendation regarding one common concern of medical students, the needle-stick injury from a patient with HBsAg-positive blood, is that both the vaccine and HBIG be given (at separate sites). Both the vaccine and HBIG should also be given to a newborn whose mother is infected with HBV. These are good examples of "passive-active" immunization, in which both immediate and long-term protection are provided.

All blood for transfusion should be screened for HBsAg. No one with a history of hepatitis (of any type) should donate blood, because non-A, non-B viruses may be present.

NON-A, NON-B HEPATITIS VIRUSES

The term "non-A, non-B hepatitis" was coined to describe the cases of hepatitis for which existing serologic tests had ruled out all known viral causes. The term is not often used because the main cause of non-A, non-B hepatitis, namely hepatitis C virus, has been identified. In addition, hepatitis D virus and hepatitis E virus have been described. Cross-protection experiments indicate additional hepatitis viruses exist.

HEPATITIS C VIRUS

Disease

HCV causes hepatitis C.

Important Properties

HCV is a member of the flavivirus family. It is an enveloped virion containing a genome of single-stranded, positive-polarity RNA. It has no virion polymerase. Multiple serotypes exist; the gene encoding the envelope glycoprotein has hypervariable regions similar to those of HIV.

Summary of Replicative Cycle

The replication of HCV is uncertain, because it has not been grown in cell culture. Other flaviviruses replicate in the cytoplasm and translate their genome RNA into large polyproteins, from which functional viral proteins are cleaved. It is likely that HCV replication follows this model.

Transmission & Epidemiology

Humans are the reservoir for HCV. It is transmitted via blood, sexually, and from mother to child. It is uncertain whether maternal transmission is across the placenta or during birth. Unlike yellow fever virus, another flavivirus that infects the liver and is transmitted by mosquitoes, there is no evidence for an insect vector for HCV.

In the United States, about 1% of blood donors have antibody to HCV. People who abuse intravenous drugs are very commonly infected. Commercially prepared immune globulin, preparations are generally very safe, but several instances of the transmission of HCV have occurred. This is the only example of an infectious disease transmitted by immune globulins.

Pathogenesis & Immunity

HCV infects hepatocytes primarily, but there is no evidence for a virus-induced cytopathic effect on the liver cells. Rather, death of the hepatocytes is probably caused by immune attack by cytotoxic T cells. HCV infection strongly predisposes to hepatocellular carcinoma, but there is no evidence for an oncogene in the viral genome or for insertion of a copy of the viral genome into the DNA of the cancer cells.

Antibodies against HCV are made, but approximately 75% of patients are chronically infected and continue to produce virus for at least a year. (Note that the rate of chronic carriage of HCV is much higher than the rate of chronic carriage of HBV.) Chronic active hepatitis and cirrhosis occur in approximately 10% of these patients. For patients who clear the infection, it is not known whether reinfection can occur or whether there is lifelong immunity.

Clinical Findings

Clinically, the acute infection with HCV is milder than infection with HBV. Fever, anorexia, nausea, vomiting, and jaundice are common. Dark urine, pale feces, and elevated transaminase levels are seen. Hepatitis C resembles hepatitis B as far as the ensuing chronic liver disease and the predisposition to hepatocellular carcinoma are concerned. Similar to HBV, a chronic - carrier state occurs with HCV. Many infections with 'HCV are asymptomatic and are detected only by the presence of antibody. The mean incubation period is 8 weeks.

Laboratory Diagnosis

HCV infection is diagnosed by detecting antibodies to HCV in an ELISA. The antigen in the assay is a recombinant protein formed from three immunologically stable HCV proteins and does not include the highly variable envelope proteins. The test does not distinguish between IgM and IgG. Because false-positive results can occur in the ELISA, a RIBA (recombinant immunoblot assay) should be performed as a confirmatory test. If the RIBA is positive, a PCR-based test that detects the presence of viral RNA in the serum should be used to determine whether active disease exists. Isolation of the virus from patient specimens is not done.

Treatment & Prevention

Alpha interferon is used for the treatment of chronic hepatitis C. It can mitigate the symptoms but does not eliminate the carrier state. Blood for transfusion is screened for the presence of HCV antibody,

which has prevented many cases of hepatitis C. There is no vaccine, and hyperimmune globulins are not available.

HEPATITIS D VIRUS (DELTA AGENT)

Disease

Hepatitis D virus (HDV) causes hepatitis D (hepatitis delta).

Important Properties & Replicative Cycle

HDV is unusual in that it is a defective virus; ie, it cannot replicate by itself, because it does not have the genes for its envelope protein. HDV can replicate only in cells also infected with HBV, because HDV uses the surface antigen of HBV (HBsAg) as its envelope protein. HBV is therefore the helper virus for HDV (Fig 41-3).

HDV is an enveloped virus with an RNA genome that is a single-stranded, negative polarity, covalently closed circle. The RNA genome of HDV is very small and encodes only one protein, the internal core protein called delta antigen. HDV genome RNA has no sequence homology to HBV genome DNA. HDV has no virion polymerase; the genome RNA is replicated and transcribed by the host cell RNA polymerase. HDV genome RNA is a "ribozyme"; ie, it has the ability to self-cleave and self-ligate, properties that are employed during replication of the genome. HDV replicates in the nucleus, but the specifics of the replicatioe cycle are complex and beyond the scope of this book.

HDV has one serotype because HBsAg has only one serotype. There is no evidence for the existence of an animal reservoir for HDV.

Transmission & Epidemiology

HDV is transmitted by the same means as is HBV, ie, sexually, by blood, and perinatally. In the United States, most HDV infections occur in intravenous drug abusers who share needles. HDV infections occur worldwide with a similar distribution to that of HBV infections.

Pathogenesis & Immunity

It seems likely that the pathogenesis of hepatitis caused by HDV and HBV is the same; ie, the virus-infected hepatocytes are damaged by cytotoxic T cells. There is some evidence that delta antigen is cytopathic for hepatocytes.

IgG antibody against delta antigen is not detected for long periods after infection, so it is uncertain whether long-term immunity to HDV exists.

Clinical Findings

Because HDV can replicate only in cells also infected with HBV, hepatitis delta can occur only in a person infected with HBV. A person can either be infected with both HDV and HBV at the same time, ie, be "coinfected," or be previously infected with HBV and then "superinfected" with HDV.

Hepatitis in patients coinfected with HDV and HBV is more severe than in those infected with HBV alone, but the incidence of chronic hepatitis is about the same in patients infected with HBV alone. However, hepatitis in chronic carriers of HBV who become superinfected with HDV is much more severe, and the incidence of fulminant, life-threatening hepatitis, chronic hepatitis, and liver failure is significantly higher.

Laboratory Diagnosis

The diagnosis of HDV infection in the laboratory is made by detecting either delta antigen or IgM antibody to delta antigen in the patient's serum.

Treatment & Prevention

Alpha interferon can mitigate some of the effects of the chronic hepatitis caused by HDV but does not eradicate the chronic carrier state. There is no specific antiviral therapy against HDV. There is no vaccine against HDV, but a person immunized against HBV will not be infected by HDV.

HEPATITIS E VIRUS

Hepatitis E virus (HEV) is a major cause of enterically transmitted hepatitis. It is a common cause of water-borne epidemics of hepatitis in Asia, Africa, India, and Mexico but is uncommon in the United States. HEV is a nonenveloped, single-stranded RNA virus tentatively classified as a member of the calicivirus family. Clinically the disease resembles hepatitis A, with the exception of a high mortality rate in pregnant women. Chronic liver disease does not occur, and there is no prolonged carrier state.

The test for HEV antibody is not readily available, so the diagnosis is typically made by excluding HAV and other causes. There is no antiviral treatment and no vaccine.

HEPATITIS G VIRUS

In 1996, hepatitis G virus (HGV) was'isolated from patients with posttransfusionhepatitis. HGV is a member of the flavivirus family, as is HCV. However, unlike HCV, which is clearly the cause of both acute hepatitis and chronic active hepatitis and predisposes to hepatocellular carcinoma, HGV has not been documented to cause any of these clinical findings. The role of HGV in the causation of liver disease has yet to be established.

RETROVIRUSES FAMILY

RNA tumor viruses have been isolated from a large number of species: snakes, birds, and mammals including nonhuman primates. The important RNA tumor viruses are listed in Table 27. They are important because of their ubiquity, their ability to cause tumors in the host of origin, their small number of genes, and the relationship of their genes to cellular oncogenes.

These viruses belong to the retrovirus family (the prefix "retro" means reverse), so named because a "reverse transcriptase" is located in the virion. This enzyme transcribes the genome RNA into double-stranded proviral DNA and is essential to their replication. The viral genome consists of two identical molecules of positive-strand RNA. Each molecule has a molecular weight of approximately 2 x 10^6 (these are the only viruses that are diploid, ie, have two copies of their genome in the virion). The two molecules are hydrogen-bonded together by complementary bases located near the 5' end of both RNA molecules. Also bound near the 5' end of each RNA is a transfer RNA (tRNA) that serves as the primer**The purpose of the primer tRNA is to act as the point Of attachment for the first deoxynuclcotidc at the start of DNA synthesis. The primers are normal-cell tRNAs that are characteristic for each retrovirus. for the transcription of the RNA into DNA. The icosahedral capsid is surrounded by an envelope with glycoprotein spikes. Some internal capsid proteins are group-specific antigens, which are common to retroviruses within a species. There are three important morphologic types of retroviruses, labeled B, C, and D, depending primarily on the location of the capsid or core. Most of the retroviruses are C-type particles, but mouse mammary tumor virus is a B-type particle, and human immunodeficiency virus (HIV), the cause of AIDS, is a D-type particle.

The gene sequence of the RNA of a typical avian sarcoma virus is gag, pol, *env,* and src. The non-transforming retroviruses have three genes; they are missing src. The gag region codes for the group-specific antigens, the pol gene codes for the reverse transcriptase, the *env* gene codes for the two envelope spike proteins, and the src gene codes for the protein kinase. In other oncogenic retro-viruses,such as HTLV-I, there is a fifth coding region (the tax gene) near the 3' end, which encodes a protein that enhances viral transcription.

The sequences at the 5' and 3' ends function in the integration of the proviral DNA and in the transcription of mRNA from the integrated proviral DNA by host cell RNA polymerase II. At each end is a sequence* called a long terminal repeat (LTR) that is composed of several regions, one of which, near the 5' end, is the binding site for the primer tRNA.

After infection of the cell by a retrovirus, the following events occur. Using the genome RNA as the template, the reverse transcriptase (RNA-dependent DNA polymerase) synthesizes doublestranded proviral DNA. The DNA then integrates into cellular DNA. Integration of the proviral DNA is an obligatory step, but there is no specific site of integration. Insertion of the viral LTR can enhance the transcription of adjacent host cell genes. If this host gene is a cellular oncogene, malignant transformation may result. This explains how retroviruses without viral oncogenes can cause transformation.

Slow Viruses

This is a heterogeneous group of agents containing both conventional viruses and unconventional agents, eg, prions. Prions are protein-containing particles with no detectable nucleic acid that are highly resistant to inactivation by heat, formaldehyde, and ultraviolet light but are killed by proteinand lipid-disrupting agents such as phenol, ether, NaOH, and hypochlorite.

In humans, the "slow" agents cause central nervous system diseases characterized by a long incubation period, a gradual onset, and a progressive, invariably fatal course. There is no antimicrobial therapy for these diseases. Note that the term "slow" refers to the disease, not to the rate of replication of the causative viruses. The replication rate of these viruses is similar to that of most other viruses.

*The length of the sequence varies from 250 to 1200 bases, depending on the virus.

HUMAN IMMUNODEFICIENCY VIRUS

Disease

Human immunodeficiency virus (HIV)* *Formerly known as human T lymphotropic virus type 3 (HTLV-III), lymphadenopathy-associated virus (LAV), and AIDS-related virus (ARV). is the cause of acquired immunodeficiency syndrome (AIDS). Both HIV-1 and HIV-2 cause AIDS, but HIV-1 is found worldwide whereas HIV-2 is found primarily in West Africa. This chapter refers to HIV-1 unless otherwise noted.

Important Properties

HIV is one of the human T cell lymphotrophic retroviruses (human T cell leukemia virus types I and II are others). HIV preferentially infects and kills helper (CD4) T lymphocytes, resulting in the loss of cell-mediated immunity and a high probability that the host will develop opportunistic infections. Other cells, eg, macrophages and monocytes, that have CD4 proteins on their surfaces can be infected also.

HIV belongs to the lentivirus subgroup of retroviruses, which cause "slow" infections with long incubation periods (see Chapter 44). HIV has a bar-shaped (type D) core surrounded by an envelope containing virus-specific glycoproteins (gpl20 and gp41) (Fig 45-1). The genome of HIV consists of two identical molecules of single-stranded, positive-polarity RNA and is said to be diploid. The HIV genome is the most complex of the known retroviruses (Fig 45-2). In addition to the three typical retroviral genes gag, pol, and env, which encode the structural proteins, the genome RNA has at least five other genes, several of which are regulatory genes.

The gag gene encodes the internal "core" proteins,, the most important of which is p24, an antigen used in serologic tests. The pol gene encodes several proteins including the virion "reverse transcriptase," which synthesizes DNA by using the genome RNA as a template, an integrase that integrates the viral DNA into the cellular DNA, and a protease that cleaves the various viral precursor proteins. The *env* gene encodes gp160, a precursor glycoprotein that is cleaved to form the two envelope (surface) glycoproteins, gp120 and gp41.

On the basis of differences in the base sequence of the gene that encodes gp 120, HIV has been subdivided into subtypes (Glades) A through I. The B Glade is the most common subtype in North America. Subtype B preferentially infects mononuclear cells and appears to be passed readily during anal sex, whereas subtype E preferentially infects female genital tract cells and appears to be passed readily during vaginal sex.

Three enzymes are located within the nucleocapsid of the virion: reverse transcriptase, integrase, and protease. Reverse transcriptase is the RNA-dependent DNA polymerase that is the source of the family name, retroviruses. This enzyme transcribes the RNA genome into the proviral DNA. Reverse transcriptase is a bifunctional enzyme; it also has ribonuclease H activity. Ribonuclease H degrades RNA when it is in the form of an RNA-DNA hybrid molecule. The degradation of the viral RNA genome is an essential step in the synthesis of the double-stranded proviral DNA. Integrase, another important enzyme within the virion, mediates the integration of the proviral DNA into the host cell DNA. The viral protease cleaves the precursor polyproteins into functional viral polypeptides.

One important regulatory gene is the tat (transactivation of transcription)' tTransactivation refers to activation of transcription of genes distant from the gene, ie other genes on the same proviral DNA or on cellular DNA. One site of action of the tat[5] protein is the long terminal repeat at: the 5' end of the viral genome. gene, which encodes a protein that enhances viral (and perhaps cellular) gene transcription. The Tat protein and another.HIVencoded regulatory protein called Nef repress the synthesis of class I MHC proteins, thereby reducing the ability of cytotoxic T cells to kill HIV-infected cells. There are several important antigens of HIV.

(1) gp120 and gp41 are the type-specific envelope glycoproteins. gp120 protrudes from the surface and interacts with the CD4 receptor (and a second protein, a chemokine receptor) on the cell surface. gp41 is embedded in the envelope and mediates the fusion of the viral envelope with the cell membrane at the time of infection. The gene that encodes gp 120 mutates rapidly, resulting in many antigenic variants. The most immunogenic region of gp120 is called the V3 loop; it is one of the sites that varies antigenically to a significant degree. Antibody against gp120 neutralizes the infectivity of HIV, but the rapid appearance of gp120 variants will make production of an effective vaccine difficult. The high mutation rate may be due to lack of an editing function in the reverse transcriptase.

(2) The group-specific antigen, p24, is located in the core and is not known to vary. Antibodies against p24 do not neutralize HIV infectivity but serve as important serologic markers of infection.

The natural host range of HIV is limited to humans, although certain primates can be infected in the laboratory. HIV is not an endogenous virus of humans; ie, no HIV sequences are found in normal human cell DNA. The origin of HIV and how it entered the human population remains uncertain. There has been speculation that monkeys or other primates were the source, but the issue is unresolved.

Viruses similar to HIV have been isolated. Examples are listed below.

(1) Human immunodeficiency virus type 2 (HIV-2) was isolated from AIDS patients in West Africa in 1986. The proteins of HIV-2 are only about 40% identical to those of the original HIV isolates. HIV-2 remains localized primarily to West Africa and is much less transmissible than HIV-1.

(2) Simian immunodeficiency virus (SIV) was isolated from monkeys with an AIDS-like illness. Antibodies in some African women cross-react with SIV. The proteins of SIV resemble those of HIV-2 more closely than they resemble those of the original HIV isolates.

(3) HTLV-IV infects T cells but does not kill them and is not associated with any disease.

Summary of Replicative Cycle

The details of the replicative cycle are incomplete, but it is thought to follow the typical retroviral cycle (see Fig 45-3). The initial step in the entry of HIV into the cell is the binding of the virion gp120 envelope protein to the CD4 protein on the cell surface. The virion gp 120 protein then interacts with a second protein on the cell surface, one of the chemokine receptors. Next the virion gp41 protein mediates fusion of the viral envelope with the cell membrane, and the virion enters the cell.

Chemokine receptors, such as fusin and CCR5 proteins, are required for the entry of HIV into CD4-positive cells. The T-cell-tropic strains of HIV bind to fusin, whereas the macrophage-tropic strains bind to CCR5. Mutations in the gene encoding CCR5 endow the individual with protection from infection with HIV. Approximately 1 % of people of Western European ancestry have homozygous mutations in this gene.

After uncoating, the virion RNA-dependent DNA polymerase transcribes the genome RNA into double-stranded DNA, which integrates into the host cell DNA. The viral DNA can integrate at different sites in the host cell DNA, and multiple copies of viral DNA can integrate. Integration is mediated by a virus-encoded endonuclease (integrase). Viral mRNA is transcribed from the proviral DNA by host cell RNA polymerase and translated into several large polyproteins, which are then cleaved by the virus-encoded protease to form the virion structural proteins, namely the reverse transcriptase, the core proteins, and the two envelope glycoproteins. The virions assemble in the cytoplasm and are released from the cell by budding. Much of the virus remains cell-associated and may be difficult to neutralize with antibody.

Transmission & Epidemiology

Transmission of HIV occurs primarily by sexual contact and by transfer of infected blood. Perinatal transmission from infected mother to neonate also occurs, either at birth or via breast milk. Infection occurs by the transfer of either HIV-infected cells or free HIV (ie, HIV that is not cell-associated). Although small amounts of virus have been found in other fluids, eg, saliva and tears, there is no evidence that they play a role in infection. In general, transmission of HIV follows the pattern of hepatitis B virus, except that HIV infection is much less efficiently transferred; ie, the dose of HIV required to cause infection is much higher than that of HBV.

Since 1981, when AIDS was first reported, and the year 1995, between 1 and 2 million people in the United States have been infected with HIV. During this time, approximately 500,000 cases of AIDS have been reported in the United States and half of those people have died. Worldwide, it is estimated that more than 30 million people are infected, mostly in Sub-Saharan Africa. Three regions, Africa, Asia, and Latin America have the highest rates of new infections.

In the United States and Europe during the 1980s, HIV infection and AIDS occurred primarily in promiscuous homosexual men, intravenous drug abusers, and hemophiliacs. Heterosexual transmission was rare in these regions in the 1980s but is now rising significantly. Heterosexual transmission is the predominant mode of infection in African countries. Very few health care personnel have been infected despite prolonged exposure and needle-stick injuries, supporting the view that the infectious dose of HIV is high. In 1990, it was reported that a dentist may have infected five of his patients. It is thought that transmission of HIV from health care personnel to patients is exceedingly rare.

Pathogenesis & Immunity

HIV infects helper T cells and kills them, resulting in suppression of cell-mediated immunity. This predisposes the host to various opportunistic infections and certain cancers such as Kaposi's sarcoma and lymphoma. However, HIV genes are not found in these cancer cells, so HIV does not directly cause these tumors. (The DNA of human herpesvirus 8 has been detected in Kaposi's sarcoma cells, but whether this virus is the cause of this cancer has yet to be determined.) HIV also infects brain monocytes and macrophages, producing multinucleated giant cells and significant central nervous system symptoms. The fusion of HIV-infected cells in the brain and elsewhere mediated by gp41 is one of the main pathologic findings. The cells recruited into the syncytia ultimately die. The death of HIV-infected cells may also be the result of immunologic attack by cytotoxic CD8 lymphocytes or antibody. Effectiveness of the cytotoxic T cells may be limited by the ability of the viral tat gene to reduce class I MHC protein synthesis.

Another mechanism hypothesized to explain the death of helper T cells is that HIV acts as a "superantigen," which indiscriminantly activates many helper T cells and leads to their demise. The finding that another retrovirus, mouse mammary tumor virus, can act as a superantigen lends support to this theory. Superantigens are described in Chapter 58.

Persistent noncytopathic infection of T lymphocytes also occurs. Persistently infected cells continue to produce HIV, which may help to sustain the infection in vivo. A person infected with HIV is considered to be infected for life. This seems likely to be the result of integration of viral DNA into the DNA of infected cells.

In 1995, it was reported that a group of HIV-infected individuals has lived for many years without opportunistic infections and without a reduction in the number of their helper T (CD4) cells. The strain of HIV isolated from them has mutations in the *nef* gene, indicating that this gene plays a role in pathogenesis.

Approximately 90% of AIDS patients have antibodies against HIV. However, these antibodies, which are detected by ELISA in the laboratory, neutralize the infectivity of the virus poorly. This indicates that immunity is incomplete and that infectious virus and antibodies can coexist.

In addition to the detrimental effects on T cells, abnormalities of B cells occur. Polyclonal activation of B cells is seen, with resultant high immunoglobulin levels. Autoimmune diseases, such as thrombocytopenia, occur.

Clinical Findings

The clinical picture of HIV infection can be divided into three stages: an early, acute stage; a middle, latent stage; and a late, immunodeficiency stage (Fig 45-4). In the acute stage, which -usually begins 2-4 weeks after infection, a mononucleosislike picture of fever, lethargy, sore throat, and generalized lymphadenopathy occurs. A maculopapular rash is also seen. Leukopenia occurs, but the number of CD4 cells is usually normal. Antibodies to HIV typically appear within 2 months after infection. Note that this delay in the appearance of antibodies can result in "false-negative" serologic tests; ie, the person is infected, but antibodies are not detectable at the time of the test.

In the middle stage, a long latent period, measured in years, usually ensues. The patient is asymptomatic during this period. Although the patient is asymptomatic and viremia is low or absent, a large amount of HIV is being produced by lymph node cells but remains sequestered within the lymph nodes. This indicates that during this period of clinical latency, the virus itself does not enter a latent state.

It is estimated that an infected person produces ,10 billion new virions each day. This viral load can be estimated by using an assay for viral RNA in the patient's plasma, and the amount of viral RNA serves to guide treatment decisions and the prognosis. For example, if a drug regimen fails to reduce the viral load, the drugs should be changed. As far as the prognosis is concerned, a patient with more than 100,000 copies of viral RNA/mL of plasma is significantly more likely to progress to AIDS than a patient with fewer than 100,000 copies.

A syndrome called AIDS-related complex (ARC) can occur during the latent period. The most frequent manifestations are persistent fevers, fatigue, weight loss, and lymphadenopathy. ARC often progresses to AIDS.

The late stage of HIV infection is AIDS, manifested by a decline in the number of CD4 cells to below 400/nun^3 and an increase in the frequency and severity of opportunistic infections.

The two most characteristic manifestations of AIDS are *Pneumocystis* pneumonia and Kaposi's sarcoma. However, many other opportunistic infections occur with some frequency. These include viral infections such as disseminated herpes simplex, herpes zoster, and cytomegalovirus infections and progressive multifocal leukoencephalopathy; fungal infections such as thrush (caused by Can*dida albicans),* cryptococcal meningitis, and disseminated histoplasmosis; protozoal infections such as toxoplasmosis and cryptosporidiosis; and bacterial infections such as disseminated *Mycobacterium avium-intracellulare* and *Mycobacterium tuberculosis* infections. Many AIDS patients have severe neurologic problems, eg, dementia and neuropathy, which can be due either to HIV infection of the brain or to many of these opportunistic organisms.

In 1992, patients with AIDS who had no evidence *of* infection by HIV-1 or HIV-2 were reported. At present, it is unknown whether another virus can cause AIDS.

Laboratory Diagnosis

The presumptive diagnosis of HIV infection is made by the detection of antibodies by ELISA. Because there are some false-positive results with this test, the definitive diagnosis is made by "Western blot" analysis, in which the viral proteins are displayed by acrylamide gel electrophoresis, transferred to

nitrocellulose paper (the blot), and reacted with the patient's serum. If antibodies are present, they will bind to the viral proteins (predominantly to the gp41 or p24 protein). Enzymatically labeled antibody to human IgG is then added. A color reaction - reveals the presence of the HIV antibody in the infected patient's serum.

HIV can be grown in culture from clinical specimens, but this procedure is available only at a few medical centers. The polymerase chain reaction (PCR) is a very sensitive and specific technique that can be used to detect HIV DNA within infected cells. Some individuals who do not have detectable antibodies have been shown by this test to be infected.

During the first month or two after infection, antibody tests are frequently negative. The presence of HIV can be detected during that period by either viral culture, p24 antigen test, or PCR assay.

Treatment & Prevention

The current treatment of choice for advanced disease is a regimen consisting of two nucleoside inhibitors and a protease inhibitor. Azidothymidine (AZT, zidovudine, Retrovir) is the nucleoside inhibitor most often used. It prolongs survival and reduces the number of opportunistic infections but does not eliminate the virus. It inhibits HIV replication by interfering with proviral DNA synthesis. However, it cannot cure an infected cell of an already integrated copy of proviral DNA. Strains of HIV resistant to AZT have been isolated from patients on long-term AZT therapy. Severe hematologic side effects can limit its use. AZT can be combined with ddI or ddC to lower the dose of each and thereby reduce the side effects.

Dideoxyinosine (ddI, didanosine, Videx) is recommended for patients who are intolerant of AZT or whose disease has progressed while they were taking AZT. Its mechanism of action is similar to that of AZT. Three other drugs, dideoxycytidine (ddC, zalcitabine, Hivid), d4T (stavudine, Zerit), and 3TC (lamivudine, Epivir) are also used in similar situations.

In addition to the nucleoside inhibitors mentioned above, there are non-nucleoside inhibitors of reverse transcriptase that are effective against HIV. Nevirapine (Viramune) is the currently approved drug in this class. The combination of nevirapine, AZT, and ddI lowers viral RNA levels and raises CD4 counts significantly more than the two-drug regimen of AZT and ddI.

Protease inhibitors, such as saquinavir (Invirase), ritonavir (Norvir), and indinavir (Crixivan), when combined with nucleoside analogues, such as AZT, are very effective in inhibiting viral replication and increasing CD4 cell counts. Mutants of HIV resistant to protease inhibitors have been isolated from patients only infrequently and are not a major clinical problem at present. Resistance to one of these drugs conveys resistance to all.

No vaccine for human use is available. A vaccine containing recombinant gp120 protects nonhuman primates against challenge by HIV and by HIV-infected cells. The success of a vaccine containing a live, attenuated mutant of SIV in protecting monkeys against challenge by a large dose of SIV may encourage a similar effort with a mutant of HIV in humans.

Prevention consists of taking measures to avoid exposure to the virus, eg, using condoms, not sharing needles, and discarding donated blood that is contaminated with HIV. Infection following a needle-stick injury may be prevented in some individuals by use of two drugs, AZT and 3TC, to which a third, indinavir, can be added. Two steps can be taken to reduce the number of HIV-infected children: AZT should be given perinatally to HIV-infected mothers and neonates, and HIV-infected mothers should not breast feed.

Several drugs are commonly taken by those in the advanced stages of AIDS to prevent certain opportunistic infections. Some examples are trimethoprim-sulfamethoxazole to prevent Pneumocystis pneumonia, fluconazole to prevent recurrences of cryptococcal meningitis, ganciclovir to prevent recurrences of retinitis caused by cytomegalovirus, and oral preparations of antifungal drugs, such as clotrimazole, to prevent thrush caused by *Candida albicans.*

33. INFECTION CONTROL PRACTICES IN THE DENTAL LABORATORY

Infection control (IC) is an essential part of dentistry. Potential for disease transmission in the dental lab is well documented. Potential pathogens can be transported to lab via orally soiled impressions, dental prostheses/appliances. Microorganisms can be transferred from contaminated impressions to dental casts. Oral bacteria can remain viable in set gypsum for up to 7 days.

Lab personnel may be exposed via:

- Direct contact (through cuts and abrasions),
- Aerosols created during lab procedures,
- Inhaled or ingested.

Patients can be at risk due to potential cross-contamination between dental prostheses/ appliances. Potential for cross-contamination from dental office to lab and back to dental office.

STANDARD PRECAUTIONS

Must be observed in the lab at all times. Are used by all lab personnel to prevent cross-contamination by dental items entering lab. All patients are treated as if they could transmit a bloodborne pathogen (BBP) disease. Examples include hepatitis B, hepatitis C, and human immunodeficiency virus (HIV).

REQUIREMENTS

Lab is responsible to comply and enforce all federal, state, and local regulations that affect its operations and employees. Includes the Occupational Safety and Health Administration's (OSHA) BBP Standard. All lab personnel: must be included in exposure determination, must be offered hepatitis B vaccine, must be given annual BBP training. Need coordination between dental office and lab. Use of proper methods/materials for handling and decontaminating soiled incoming items are needed. All contaminated incoming items should be cleaned and disinfected before being handled by lab personnel, and before being returned to the patient.

"BARRIER" SYSTEM

Is most effective, practical method for preventing cross-contamination. This system represents a series of physical cleaning procedures to reduce organic debris and microorganisms on intraorally soiled dental items. It is accomplished through step-wise process of mechanical and chemical cleaning and disinfection. It results in a product that can safely be handled by lab personnel without need for personal protective equipment (PPE).

This system includes:

- Handwashing with plain or antimicrobial soap (or an alcohol-based hand rub if hands are not visibly soiled).
- Use of PPE when there is potential for occupational exposure to BBPs. Examples: gloves, mask, protective eyewear, chin length face shield, protective clothing (i.e., lab coat/jacket).

Figure 3. Personal protective equipment (PPE)

GLOVES

- Disposable gloves
  - Use when there is potential for direct hand contact with contaminated items
  - Should be changed and disposed of appropriately after completion of procedure
  - Hands should be washed before gloving and after removing gloves
- Utility gloves
  - Should be used when cleaning/disinfecting equipment/surfaces

MASK/PROTECTIVE EYEWEAR/CLOTHING

Must be used when there is potential for splashes, spray, spatter, or aerosols. Examples: when operating lathes, model trimmers, and other rotary equipment. Lab coat/jacket should be worn at all times during fabrication process. They must be changed daily. Do not wear outside of the lab. The lab coats should be launder appropriately.

CHEMICAL DISINFECTANTS

- Two functions
  - Must be an effective antimicrobial agent
  - Must not adversely affect dimensional accuracy or surface texture of impression materials and resulting gypsum cast.

We use disinfectants because we want to reduce likelihood of ill fitting, nonfunctional prostheses.

All employees must be properly trained to handle these materials in accordance with OSHA's Hazard Communication Standard. Disinfectant must have an Environmental Protection Agency (EPA) registration number. Must have at least intermediate-level of activity.

INCOMING ITEMS

- Rinse under running tap water to remove blood/saliva
- Disinfect as appropriate
- Rinse thoroughly with tap water to remove residual disinfectant
- No single disinfectant is ideal or compatible with all items

- Annotate the DD Form 2322: *"Disinfected with _____ for _____minutes"*

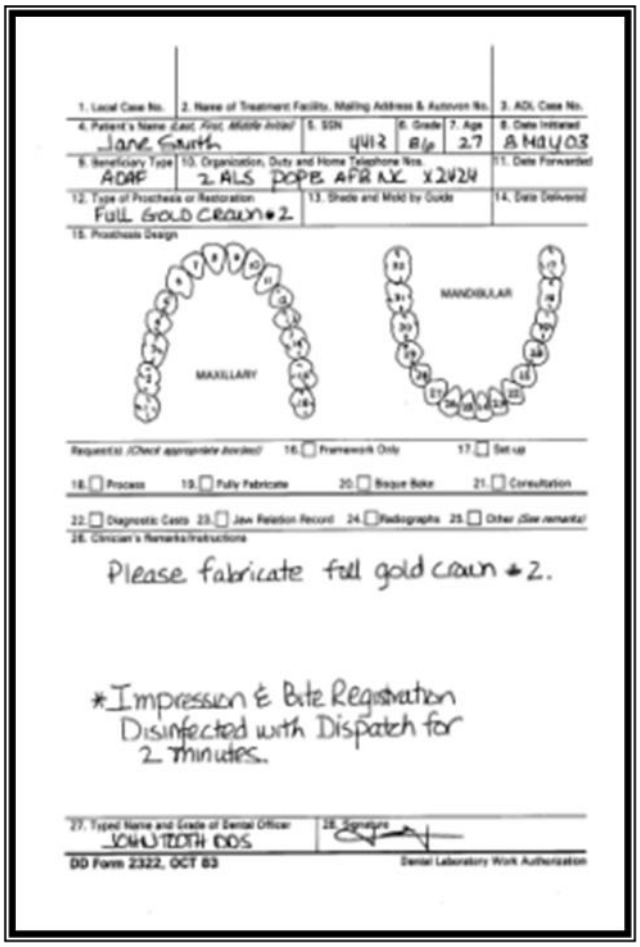

Figure 4. The DD Form

OUTGOING ITEMS

- Clean and disinfect before delivery to patient
- After disinfection: rinse and place in plastic bag with diluted mouthwash until insertion
- Do not store in disinfectant before insertion
- Label the plastic bag: *"This case shipment has been disinfected with _____ for _____ minutes"*

Figure 5. Outgoing Items

IMPRESSIONS

- Many studies have been performed to evaluate effects of various disinfectants on different types of impression materials
- Research findings have been contradictory
- No single disinfectant is compatible with all impression materials
- The least distortion is associated with products having the shortest contact times

Figure 6. Impression

- Many variables can affect impression materials
  - Composition and concentration of disinfectants
  - Exposure time and compatibility of various disinfectants with specific impression materials
  - Physical/chemical properties can vary in a given category of material or disinfectant

- Do an in-office "test run" when using new combinations of impression materials and disinfectants
- Consult dental materials' manufacturers regarding their compatibility with disinfectants

Disinfecting Impressions

- Methods
  - Spraying, dipping, immersing
- Exposure time should be that recommended by the manufacturer of disinfectant for tuberculocidal disinfection
- Iodophors, sodium hypochlorite (1:10 concentration), chlorine dioxide, phenols, and other approved products are all acceptable
- Polyether materials cannot be immersed in disinfectants due to potential for absorption and distortion
- Immersion disinfectants can only be used once before discarding (except for glutaraldehydes)
- Most reports indicate dimensional stability is not significantly affected by immersion technique
- Clean and rinse impression in dental operatory
  - Cleaning efficiency can be improved by gently scrubbing impression with camel's hair brush and antimicrobial detergent
- Sprinkle dental stone into impression before rinsing to aid in cleaning
- Cleaning and rinsing
  - Reduces bioburden present
  - Lessens overall microbiologic challenge to disinfectant
- Spray, dip, or immerse impression in appropriate intermediate- or high-level disinfectant and place in sealed bag
- Disinfection can be accomplished in the dental operatory or a professional work area depending on facility policy
- After required contact time, rinse impression and pour-up

Spray Technique

- Rinse entire impression/tray under running tap water after removal from oral cavity
- Trim excess impression material from noncritical areas
  - Reduces number of microorganisms and organic debris present
- Place impression in bag and liberally spray the entire impression/tray
- Seal bag to create "charged atmosphere"
  - Reduces exposure to vapors and liquid
- Remove from bag at end of exposure time; rinse and pour
- Once stone has set, remove cast from impression
- Dispose of impression material and disposable tray (if applicable) in general waste
- Sterilize reusable tray (if applicable)

Advantages

- Uses less disinfectant
- Same disinfectant can often be used to disinfect environmental surfaces

Disadvantages

- Probably not as effective as immersion

- Can be released into air increasing occupational exposure

DENTAL CASTS

- Very difficult to disinfect
- Is preferable to disinfect impression
- If casts must be disinfected:
  - Place casts on end to facilitate drainage
  - Spray with iodophor or chlorine product, then rinse
- Another option
  - Soak casts for 30 minutes in 0.5% concentration of sodium hypochlorite and saturated calcium dihydrate solution (SDS)
  - SDS is produced by placing uncontaminated, set gypsum (i.e. stone) in a container of water

ORALLY SOILED PROSTHESES

- Scrub with brush and antimicrobial soap to remove debris and contamination
  - Can be accomplished in operatory or professional work area
  - Sterilize brush or store in approved disinfectant
- Place prosthesis in sealable plastic bag or beaker filled with ultrasonic cleaning solution or calculus remover
- Place in ultrasonic cleaner for required time as specified by manufacturer of ultrasonic cleaner
- Place cover on ultrasonic cleaner to reduce spatter potential
- Remove and rinse under running tap water, dry, and accomplish required work

Sub-Surface Disinfection

- Place prosthesis in sealable plastic bag containing 1:10 dilution of sodium hypochlorite or other intermediate- to high-level disinfectant (not glutaraldeyde or phenols)
- Place in ultrasonic cleaner for 10 minutes
- Do not exceed manufacturer's recommended contact time on metal components to minimize corrosion
- There is little effect on chrome-cobalt alloy with short-term exposures (10 minutes)
- Do not store in disinfectant before insertion

Store in diluted mouthwash until insertion

WASTE

- Can include disposable trays, impression materials, and contaminated packing materials (if cannot be disinfected)
- Dispose of according to applicable federal, state, and local regulations
- Dispose of in general waste unless defined as regulated waste
- Only small amounts of regulated waste are generated in lab
- Sharps should be placed in puncture-resistant container
- Ways to reduce risk of injury from aerosols, spatter, and macroscopic particles
  - Use protective eyewear
  - Ensure plexiglass shield is in position
  - Activate vacuum
- Pumice has been shown to pose a potential contamination risk
  - Via aerosol or direct contact

- Mix pumice with
  - Clean water, diluted 1:10 bleach, or other appropriate disinfectant

Add tincture of green soap if desired

- Change pumice daily
- Machine should be cleaned and disinfected daily
- No need for separate pans for new and existing prostheses if isolated properly
- At a minimum clean and disinfect pumice brushes and rag wheels daily. Daily heat sterilization is preferable.

STERILIZATION

- Heat sterilize all metal and heat-stable instruments that contact oral tissues, contaminated appliances, or potentially contaminated appliances should be heat sterilized after each use
  - Examples: facebow fork, metal impression trays, burs, polishing points, rag wheels, laboratory knives.

IMPRESSION TRAYS

- Precleaning removes bioburden and any adherent impression material
- Ultrasonic cleaning can aid in removing residual set gypsum
- Chrome-plated or aluminum trays
  - Clean, package, heat sterilize
- Single-use trays
  - Discard after one use
- Custom acrylic trays
  - Can be disinfected (by spray or immersion), then rinsed (if to be used for second appointment)

DISINFECTION

- Prosthodontic items contaminated by handling should be disinfected (by spray or immersion technique based on type of item) after each use
  - Examples: alcohol torch, facebow, articulator, mixing spatula, mixing bowl, lab knife, shade/mold guide

WAX BITES/RIMS, BITE REGISTRATIONS

- Immersion disinfection may cause distortion to some items
  - Use spray disinfection
- Heavy-body bite registration materials
  - Usually not susceptible to distortion and can be disinfected in same manner as an impression of the same material

LAB EQUIPMENT

- Follow manufacturer instructions for:
  - Maintenance
  - Cleaning
  - Disinfection
  - Compatibility with disinfectants

ENVIRONMENTAL SURFACES

- Disinfection procedures should be comparable to procedures performed in the operatory
- Clean and disinfect daily or when visibly contaminated
- Use EPA-registered, tuberculocidal, hospital-grade disinfectant according to manufacturer instructions
  - Use utility gloves
- May use surface barriers to reduce the need to use disinfectants

PERSONAL HYGIENE

- Refrain from the following activities while in the lab where there is potential for occupational exposure:
  - Eating
  - Drinking
  - Smoking
  - Applying cosmetics or lip balm
  - Handling contact lenses

SPECIAL CONSIDERATIONS

- For porcelain restorations that are characterized intraorally
  - Take them directly to porcelain furnace
  - Sintering process sterilizes restoration
  - No need for separate cleaning/disinfection process
  - Monitor procedures closely to ensure proper cleaning/disinfection of equipment and areas that may become contaminated during the process

34. NORMAL MICROFLORA OF THE ORAL CAVITY

34.1 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 28. 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*105	100
S. salivarius	100	10'	100
S. mitis	100	10M08	100
Saprophytic neisseriae	100	10s-10''	-ы-
Lactobacilli	90	lo'-io''	**+**
Staphylococci	80	юМо''	*++*
Diphtheria-like corynebacteria	80	No data	*+*
Actinomycetes	100	No data	++
Candida and other yeast-like fungi	50	10M01	+
Mycoplasmas	10s-10!	No data	
II. Obligate anaerobes			
Veilonellas	100	106-10*	100
Anaerobic streptococci (peptostreptococci)	100	No data	100
Бактероиды	100	No data	100
Fusobacteria	75	lOMO4	100
Group B. Transient allochthonous microflora			
Aerobic and facultatively			
anaerobic:			
Gram-negative rods			
Klebsiella spp.	15	10-102	0
Aerobacter spp.	3	10-102	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 grampositive 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. Nonsoluble 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 C02 and H20 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; produce 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.

34.2. 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 become 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 number 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

34.3. 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 min to more than 5 billion per 1 ml, for about 750 min 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 105-106 of bacterial cells per 1 cm2. 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 103 to 106 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.

35. INFLAMMATION AND PATHOLOGY OF THE ORAL CAVITY

35.1 MICROBIOLOGY OF 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:

Dl. 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.

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.

35.2 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 a-hemolytic streptococci, representatives of gram-negative nonsporeforming 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.

35.3 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.

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 *Veilloneila, Wolinella, Capnocyiophaga* 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 1 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.

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.

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 107-109 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. viscosits)* 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, Aggregatihacter, 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.

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 29).

Table 29. 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	

35.4 DENTOALVEOLAR INFECTIONS

Dentoalveolar infections can be defined as *pyogenic* infections associated with the tooth and its surrounding supporting structures, such as the periodontium and the alveolar bone. These infections can (also be known as and) include the following:

- **periapical abscess**
- **apical abscess**
- **chronic periapical dental infection**
- **dental pyogenic infection**
- **periapical periodontitis**
- **dentoalveolar abscess.**

The **clinical presentation** of dentoalveolar infections depends on: the virulence of the causative microorganisms, the local and systemic defense mechanisms of the host, and the anatomical features of the region.

Depending on the interactions of these factors, the resulting infection may present as:

- a local abscess surrounding the diseased tooth
- a diffuse cellulitis that spreads along fascial planes
- a combination of the conditions listed above

Pathogenesis

The source of the infection are usually endogenous oral commensals, usually from the apex of a necrotic tooth or from the periodontal pockets as a result of either caries or periodontal disease. The microorganisms can invade the *pulp* and *periapical tissue* from the **apical foramen, via the periodontal ligament, or via blood** (Figure 7).

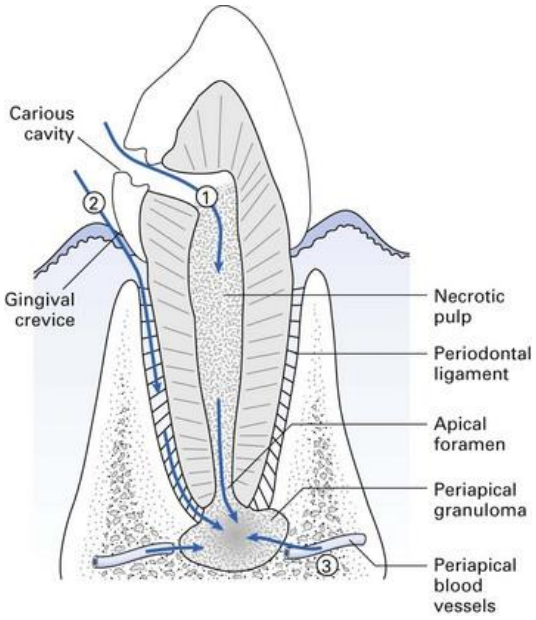

Figure 7 - Pathogenesis of dentoalveolar infections (1) apical foramen (2) periodontal ligament (3) blood

A dentoalveolar **abscess** usually develops by the extension of the initial carious lesion into dentine, where bacteria spread to the pulp via the *dentinal tubules*. The pulp responds to the infection either by rapid **acute inflammation** involving the whole pulp, which quickly becomes necrotic, or by development of a **chronic localized abscess** with most of the pulp remaining viable. Other ways in which microbes reach the pulp are shown in (Figure 8) below:

- by tooth fracture or pathological tooth wear
- by traumatic exposure during dental treatment (iatrogenic)
- through the periodontal membrane (periodontitis and pericoronitis) and accessory root canals.
- rarely by **anachoresis**, i.e. seeding of organisms directly into pulp via the pulpal blood supply during bacteremia (e.g. tooth extraction at a different site).

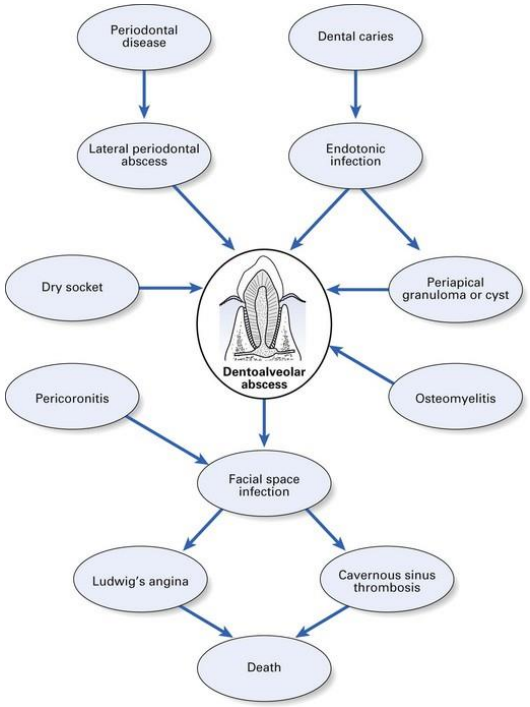

Figure 8 – Additional causes that may result in dentoalveolar abscess

Once **pus** formation occurs, it may remain:

- **localized at** the root apex, where it can develop into an **acute** or **chronic** abscess
- develop into **focal osteomyelitis**
- or spread into **the surrounding tissue**

Microbiologically, the *dentoalveolar abscess* is characterized by the following features:

- **polymicrobial** (endogenous) infection (more frequent), with a mixture of three or four different species.
- **monomicrobial** (endogenous) infection (with a single organism) is unusual.
- strict anaerobes are the predominant organisms, with *S. Viridans* a group being less commonly found than once believed.

Common species isolated from the abscesses are: *Prevotella*, *Porphyromonas*, *Fusobacterium*, and anaerobic *Streptococci*. Facultative anaerobes like *Streptococcus milleri* are the second largest group. There is evidence that some strictly anaerobic bacteria, especially *Porphyromonas gingivalis* and *Fusobacterium* are more likely to cause a more severe infection than other species, and that synergistic microbial interactions play an important role in the severity of dentoalveolar abscesses. See more examples in (Table 30) below:

Table 30. Bacteria commonly isolated from dentoalveolar abscesses

Facultative anaerobes	**Obligate anaerobes**
Streptococcus milleri	*Peptostreptococcus* species
Streptococcus sanguinis	*Porphyromonas gingivalis*
Actinomyces species	*Prevotella intermedia*
	Prevotella melaninogenica
	Fusobacterium nucleatum

DIRECT SPREAD

(1) Spread into the superficial soft tissues may:

- **localize** as a soft-tissue abscess.
- extend through the overlying oral mucosa or skin, producing a **sinus** linking the main abscess cavity with the mouth or skin.
- extend through the soft tissue, resulting in **cellulitis**.

(2) Spread may occur into the adjacent fascial spaces, following the **path of least resistance**; this depends on the anatomical relation of the original abscess to the adjacent tissues. Infection via fascial planes often spreads rapidly and for some distance from the original abscess site, and occasionally may cause severe respiratory distress as a result of occlusion of the airway by edema (Ludwig's angina).

(3) Infection may extend into the deeper medullary spaces of alveolar bone, producing a spreading **osteomyelitis**. This may occur in immunocompromised patients.

(4) In maxillary teeth, odontogenic infection may directly spread into the maxillary sinus, especially if the sinus lining and the tooth apex are subjacent, leading to acute or chronic **secondary maxillary sinusitis** (as opposed to primary sinusitis due to direct sinus infection). Such infection, if not arrested, may rarely spread to the central nervous system, causing serious complications such as **subdural empyema, brain abscesses or meningitis.**

INDIRECT SPREAD

• **lymphatic routes**: to regional nodes in the head and neck region (submental, submandibular, deep cervical, parotid and occipital). The involved nodes are tender, swollen, and painful, and rarely may suppurate, requiring drainage.

• **hematogenous routes**: to other organs such as the brain (rare).

Clinical signs and symptoms depend on the:

• site of infection
• degree and mode of spread
• virulence of the causative organisms
• efficiency of the host defense system

Clinical manifestation may include a non-viable tooth with or without a carious lesion, a large restoration, evidence of trauma, swelling, pain, redness, trismus, local lymph node enlargement, sinus formation, raised temperature and malaise. The last two symptoms are a direct consequence of increased levels of systemic inflammatory cytokines such as interleukins and tumor necrosis factor in response to bacterial products such as lipopolysaccharides (endotoxins).

Whenever possible, **pus** should be collected by **needle aspiration.**

35.5 INFLAMMATION OF THE ORAL MUCOSA

Inflammations of the **oral mucosa** include the following:

- **Glossitis** - inflammation of the tongue
- **Stomatitis** - inflammation of larger parts of the oral mucosa
- **Gingivitis** - inflammation of the gum
- **Cheilitis** - inflammation of the lips

Abscesses and phlegmon can be observed when deeper parts of the oral mucosa are involved.

The **most common causes** of inflammation are:

- **trauma** from ill-fitting dentures or braces, biting the inside of the cheek, tongue, or lip, and surgery
- chemotherapy treatment

- viral infection (e.g herpes)
- yeast infection (e.g thrush)
- any condition associated with xerostomia (dry mouth)
- smoking or chewing tobacco

Other examples include:

- bacterial infections
- sexually transmitted infections
- weakened or deficient immune system
- irritation from strong chemicals
- stress
- certain diseases, including Behcet's disease, Crohn's disease, and Lupus
- medications, including sulfa drugs, anti-epileptics, and some antibiotics
- nutritional deficiencies
- allergic reactions
- burns caused by hot food and drink

It is important to identify the **cause** of inflammation in order to accord proper treatment.

A. GLOSSITIS

Glossitis refers to inflammation of the tongue. The tongue is the small, muscular organ in the mouth that helps you chew and swallow food, as well as talk. The condition causes the tongue to swell in size, change in color, and develop a different appearance on the surface. Glossitis may cause the small bumps on the surface of the tongue (**papillae**) to disappear. The papillae contain thousands of tiny sensors called **taste buds** and play a role in how you eat. Severe tongue inflammation that results in swelling and redness can cause pain and may change the way you eat or speak.

Types of glossitis

Glossitis can take the following forms:

- **Acute glossitis** - an inflammation of the tongue that appears suddenly and often has severe symptoms, like during an *allergic reaction.*
- **Chronic glossitis-** an inflammation of the tongue that continues to recur. This type may begin as a symptom of another health condition.
- **Atrophic glossitis-** also known as Hunter glossitis, occurs when many papillae are lost. This results in changes in the tongue's color and texture. This type of glossitis typically gives the tongue a glossy appearance.

Causes

The following factors can result in inflammation of the tongue:

- **Allergic reactions -** to medications, food, and other potential irritants may aggravate the papillae and the muscle of the tongue. (examples of irritants: toothpaste, certain high blood pressure medication)
- **Diseases-** certain diseases that affect your immune system may attack the tongue's muscles and papillae. *Herpes simplex*, a virus that causes cold sores and blisters around the mouth, may contribute to swelling and pain in the tongue.
- **Low iron levels**
- **Mouth trauma-** cuts and burns on the tongue or dental appliances like braces placed on your teeth.
- **Chronic *Candida* infections or tertiary syphilis (*Treponema pallidum)***

Risk Factors

A patient may be at risk for glossitis if they:

- have a mouth injury
- eat spicy foods
- wear braces or dentures that irritate their tongue
- have herpes
- have low iron levels
- have food allergies
- have an immune system disorder

Clinical Manifestation

General symptoms include:

- pain or tenderness in the tongue
- swelling of the tongue
- change in the color of the tongue
- an inability to speak, eat, or swallow
- loss of papillae on the surface of the tongue

Diagnosis

Usually consists of an assessment by a dentist or a physician. Samples of saliva and blood may also be taken and sent to a laboratory for further examination.

Treatment

Treatment for glossitis typically includes a combination of medications and home remedies.

- **Medications-** Antibiotics and other medications that get rid of infections may be prescribed if bacteria are present in your body. The physician may also prescribe topical corticosteroids to reduce the redness and soreness.
- **Home care-** Brushing and flossing teeth several times a day may improve the health of the tongue, gums, and teeth. This can help relieve the symptoms associated with glossitis and prevent the condition from recurring.

Prognosis

In most cases, glossitis goes away with time or treatment. Treatment may be more successful if one avoids foods that cause inflammation of the tongue. Practicing proper oral hygiene may also help reduce or prevent problems.

B. STOMATITIS

Stomatitis is a type of **mucositis**, a condition defined as pain or inflammation of the oral mucosa, which are the thin skin coverings on the inside surface of the mouth. The membranes produce protective mucus, as well as lining the digestive system, from the mouth to the anus. Stomatitis can be caused by a variety of different factors, which may overlap with each other at the same time. These **factors** can include: injury, infection, allergy, or skin disease.

There are two **most common** types of stomatitis are the following:

Canker sores

Canker sores, also known as **apthous ulcers**, are one of the most common cause of stomatitis. They usually develop on the inside of the lips or cheek and are pale white or yellow in color with an outer red ring. They can be found as a single lesion or as a cluster. Canker sores lead to acute pain. In most (usually minor) cases, the ulcers heal within 4-14 days. In more severe cases, which account for about **1 in 10** of all cases of stomatitis, the sores can last up to 6 weeks. Anyone can get canker sores, although women and people in their teens and 20s are more likely to experience them. They can have a genetic tie, however, they are not contagious.

Cold sores

Cold sores are small, painful, fluid-filled sores that usually occur on or around the lips, near the edge of the mouth. Caused by the **herpes** virus (HSV), the condition is also known as ***herpes stomatitis***. A person may experience a tingling or burning sensation before the sore appears, as well as tenderness. Cold sores dry up and crust over with a yellow-colored scab. Cold sores tend to last for around 5-7 days and tend to be recurrent. These types of lesions are contagious.

Clinical Manifestation

- mouth ulcers with a white or yellow layer and red base, usually inside the lips, cheek, or on the tongue
- red patches
- blisters
- swelling
- oral dysaesthesia – a burning feeling in the mouth
- lesions that heal in 4-14 days and often recur

Diagnosis

Diagnosis depends on the cause of the stomatitis, but is usually done via physical examination by a physician. A physician can also look at a person's medical history to see if a current or previous medication has caused the stomatitis. The doctor will also ask the patient about their sexual history and whether they have ever smoked.

Other **tests** might include:

- **swabs,** both bacterial and viral
- **tissue scrapings or swabs** for fungal infections
- **biopsy**, or the removal of cells or tissue for further study
- **blood tests**
- **patch tests** to identify allergy

Treatment

Treatment for stomatitis will also depend on the cause. Treating the root cause is important for stomatitis caused by the following:

- Allergy
- Infection
- Disease
- Nutritional deficiency

Topical treatment

Topical treatments applied directly to the skin have been found to help lessen the pain and speed up the healing process. Types of topical treatment include:

- **Topical corticosteroids**: Often a rinse format, these aim to eliminate symptoms to allow the person to eat, drink, and speak without pain or discomfort.
- **Topical antibiotics**: usually in gel or rinse format and have anti-inflammatory and antibiotic properties.
- **Topical anesthetics**: numbing medications, mostly available by prescription that people can apply directly to the sores for temporary pain relief.
- **Kanka**: An over-the-counter product that provides a barrier layer to mouth sores, giving temporary pain relief.

Prevention

The following precautions can be taken to help a patient prevent recurrence:

- using an antiseptic and non-alcoholic mouthwash
- treating chronic dry mouth

- using a soft toothbrush
- maintaining proper nutrition and hydration
- receiving routine dental care

C. CHEILITIS

Cheilitis is inflammation of the lips. This inflammation may include the **perioral** skin (the skin around the mouth), the **vermilion border**, or the **labial mucosa** of various etiologies, which occurs relatively often. The disease may appear as an isolated condition or as part of certain systemic conditions. Cheilitis can co-occur with many conditions including **anemia, oral candidiasis, atopy, contact reaction to an irritant or allergen (to cosmetics), drug intake (retinoids), etc**. Generally, the most commonly reported forms in the literature are **angular, contact (allergic and irritant), actinic, and exfoliative cheilitis.**

Types:

Cheilitis can present in the following forms:

- cheilitis simplex (common cheilitis, "chapped lips")
- actinic cheilitis – long term exposure to ultraviolet radiation
- angular cheilitis – nutritional deficiency (vitamin B, folate), edentulism, *Candida albicans, Staphylococcus aureus, Beta hemolytic Streptococcus*
- eczematous cheilitis
- infectious cheilitis- *Candida, Staphylococcus aureus, Herpes simplex, Streptococcus pyogenes*
- granulomatous cheilitis
- drug-related cheilitis
- exfoliative cheilitis
- cheilitis grandularis
- plasma cell cheilitis

or can be due to **other causes** like:

- Lupus erythematosus- lupus cheilitis
- Crohn's disease (angular cheilitis)
- "Nutritional cheilitis"- pyridoxine (vitamin B6) deficiency.
- Lichen planus
- Pemphigoid
- Xerostomia

Diagnosis

A complete examination of the patient's oral cavity, skin, and other mucosa by the physician. When approaching patients, several factors need to be taken into account, particularly patient general medical history (existence of diabetes, atopy, immunosuppression), exposure to external factors (eweather conditions), the possibility of vitamin or mineral deficiencies, undesirable habits (lip licking, frequent sun exposure, lip contact with various substances), etc. It is also important to know whether the lesions are **persistent** or whether they are reversible. Some lip lesions require **biopsies**, such as chronic actinic cheilitis (to examine for severe dysplasia or cancer) or granulomatous cheilitis (to confirm the diagnosis)

Treatment

The following are some examples of treatment options for cheilitis, depending on the cause:

- Lip balm or thick emollient ointment, applied frequently.
- Topical antiseptics.
- Topical or oral anti-staphylococcal antibiotic.
- Topical antifungal cream.
- Oral antifungal medication.

- Topical steroid ointment.
- Nutritional supplements.

35.6 INFLAMMATION OF THE SALIVARY GLANDS

The salivary glands are in charge of making saliva and releasing it into the mouth. Saliva helps with swallowing and digestion and protects your teeth from bacteria.

There are **three pairs** of **major** salivary glands (**Figure 9**):

- **Parotid glands.** Located in the upper part of each cheek, close to the ear. The duct of each parotid gland empties onto the inside of the cheek, near the molars of the upper jaw.
- **Submandibular glands.** Under the jaw. They have ducts that empty behind the lower front teeth.
- **Sublingual glands.** Beneath the tongue. They have ducts that empty onto the floor of the mouth.

Figure 9 - Types of major salivary glands

In addition to these major glands, 600 to 1,000 very tiny, **minor** salivary glands are scattered throughout the mouth and throat. They are located under the mucosa that lines the:

- Inner lips
- Inner cheeks
- Palate
- Back of the throat
- Back portion of the tongue
- Pharynx
- Sinuses

Some of the **most common salivary gland** disorders include:

- **Sialolithiasis** (salivary gland stones).Tiny, calcium-rich stones sometimes form inside the salivary glands. The exact cause of these stones is unknown.
- **Sialadenitis** (infection of a salivary gland). Sialadenitis is a painful infection that usually is caused by bacteria.
- **Viral infections.** Systemic viral infections sometimes settle in the salivary glands. This causes facial swelling, pain and difficulty eating. (ie. Mumps)
- **Cysts**-Babies sometimes are born with cysts in the parotid gland because of problems related to ear development before birth. Later in life, other types of cysts can form in the major or minor salivary glands. They may result from traumatic injuries, infections, or salivary gland stones or tumors.
- **Benign tumors**
- **Malignant tumors**
- **Sjogren's syndrome**.

- **Sialadenosis** (nonspecific salivary gland enlargement). Sometimes, the salivary glands become enlarged without evidence of infection, inflammation, or tumor. This nonspecific enlargement is called sialadenosis. It most often affects the parotid gland, and its cause remains unknown.

SIALADENITIS

Sialadenitis refers to a **rare** condition that causes inflammation of a salivary gland. The condition is most common among elderly adults and mostly affects the **parotid and submandibular glands.** It can be an **acute, chronic, or recurrent condition**.

Sialadenitis is most common among elderly adults with salivary gland stones, calcified structures that can form inside a salivary gland and block the flow of saliva into the mouth. Sialadenitis can also occur in other age groups, including infants during the first few weeks of life. It can also often happen in people who are sick or recovering from surgery, or people who are dehydrated, malnourished, or immunocompromised. It is usually caused by a virus or bacteria, commonly as a result of poor oral hygiene.

Predisposing Factors:

- sialolithiasis
- decreased flow (dehydration, post-operative, drugs)
- poor oral hygiene
- exacerbation of low grade chronic sialoadenitis

Clinical manifestation:

- Enlargement, tenderness, and redness of one or more salivary glands
- Fever (when the inflammation leads to infection)
- Decreased saliva (in both acute and chronic sialadenitis)
- Pain while eating
- Dry mouth (xerostomia)
- Reddened skin
- Swelling in the cheek and neck region

Acute sialadenitis can result from both bacterial infections (retrograde spread of bacteria secondary to decreased salivary flow) and viral infections (mumps, cytomegalovirus).

Chronic sialadenitis can develop secondary to ductal obstruction (sialolithiasis) or in certain immune-related disorders, such as Sjögren syndrome and sarcoidosis.

Bacterial sialadenitis:

Sialadenitis caused by bacteria is a rare condition that can be attributed to the ascending infection of ductal system by *Staphylococcus aureus*, *Streptococcus viridans* or gram negative bacteria (Table 31).

Bacterial sialadenitis most commonly affects the **parotid gland** and presents as a painful swelling of the cheek that may be associated with trismus and low-grade fever. When the gland is massaged, a purulent exudate may be expressed from the parotid duct. Such infections sometimes develop after a surgical procedure and are therefore known as **"surgical mumps."** Because fluid intake is suppressed prior to surgery and atropine is given during surgery, patients have decreased salivary flow and are more susceptible to a retrograde bacterial infection.

Table 31. Species of bacteria that can cause sialadenitis

Aerobe species	**Anaerobe species**
Staphylococcus aureus	*Prevotella*
Haemophilus influenzae	*Porphyromonas species*
	Fusobacterium species
	Peptostreptococcus species

Chronic lymphoepithelial sialadenitis- "LESA"

A relatively common chronic asymptomatic lymphocytic inflammation. It is often associated with obstruction (atrophy and fibrosis), rheumatoid arthritis, Sjögren syndrome, sialolithiasis, and mumps.

50% are monoclonal by PCR but MALT lymphoma has ducts surrounded by broad coronas of monocytoid cells, infiltration of interfollicular region by monocytoid cells or atypical plasma cells containing Dutcher bodies, monoclonality by immunohistochemistry or flow cytometry, monocytoid infiltrates in regional lymph nodes.

Chronic sclerosing sialadenitis:

Most patients are male and over 50 years of age. 40% of cases are associated with a history of allergic disease such as bronchial asthma or chronic sinusitis.Serum IgG4 concentration is dramatically elevated in many patients but may be normal in up to 40%. Characterized microscopically by the presence of three **major** criteria:

- dense lymphoplasmacytic infiltrate
- storiform pattern of fibrosis
- obliterative phlebitis

Minor criteria are phlebitis without obliteration of the lumen and increased numbers of eosinophils.

In the absence of **less than two of the major** criteria, elevated tissue IgG4: IgG ratio (> 40%) or elevated serum IgG4 (> 135 mg/dl) may corroborate diagnosis.

Sclerosing polycystic sialadenitis:

A rare tumorous condition predominantly affecting the parotid gland (in 80% of cases).In some cases, it may be considered a neoplasm due to monoclonal nature of the lesion. May recur after resection (20%) due to multifocal disease / incomplete resection. Generally, it is not associated with malignant transformation.

Diagnostic tests:

- **Culture and sensitivity testing** of exudate from *salivary duct.* Culturing of **purulent discharge** is advisable in acute presentations of sialadenitis to allow targeted antibiotic therapy.
- **Full blood count** if infection is suspected.
- **Facial radiographs** such as *dental radiographic* views should be taken to exclude an obstructive element due to presence of sialolith or evolving abscess. However, sialoliths with low calcium phosphate content may not be visible.

Treatment

Sialadenitis is usually first treated with an antibiotic after the appropriate culture is performed. Examples of antibiotics prescribed usually cover oral flora, like that of **amoxicillin/clavulanate** or **clindamycin**. **Other** treatments exist that can help with the pain and increasing saliva flow. These include drinking lemon juice or sucking hard candy, using warm compresses, and gland massages.

Without proper treatment, sialadenitis can develop into a **severe infection**, especially in elderly or immunocompromised people. Sometimes the inflammation/ infection can progress into an **abscess,** which needs to be drained. In rare cases, **surgery** may also be needed.

Complications may include: abscess, dental decay, and postparotidectomy complications (facial deformity or facial nerve palsy).