

Practical lesson 20 : Introduction to special virology. Microbiological diagnosis of acute respiratory viral infections (family *Orthomyxoviridae*, *Paramyxoviridae*. *Adenoviridae*, *Coronaviridae*). The role of poxviruses in human pathology

ORTHOMYXOVIRUSES

INFLUENZA VIRUSES

Influenza viruses are important human pathogens because they cause both outbreaks of influenza that sicken and kill thousands of people each year as well as infrequent but devastating worldwide epidemics (pandemics). Influenza viruses are the only members of the ortho-myxovirus family. The orthomyxoviruses differ from the paramyxoviruses primarily in that the former have a segmented RNA genome (usually eight pieces), whereas the RNA genome of the latter consists of a single piece. The term myxo refers to the observation that these viruses interact with mucins (glycoproteins on the surface of cells).

In addition, the orthomyxoviruses are smaller (110 nm in diameter) than the paramyxoviruses (150 nm in diameter).

In 1997, an outbreak of human influenza (avian influenza, bird flu) caused by an H5N1 strain of influenza A virus began. In 2009, there was an outbreak of human influenza caused by H1N1 influenza A virus of swine origin (swine-origin influenza virus, S-OIV). In 2013, an outbreak of influenza caused by an H7N9 strain of influenza virus occurred.

1. Human Influenza Virus

Disease

Influenza A virus causes worldwide epidemics (pandemics) of influenza, influenza B virus causes major outbreaks of influenza, and influenza C virus causes mild respiratory tract infections but does not cause outbreaks of influenza.

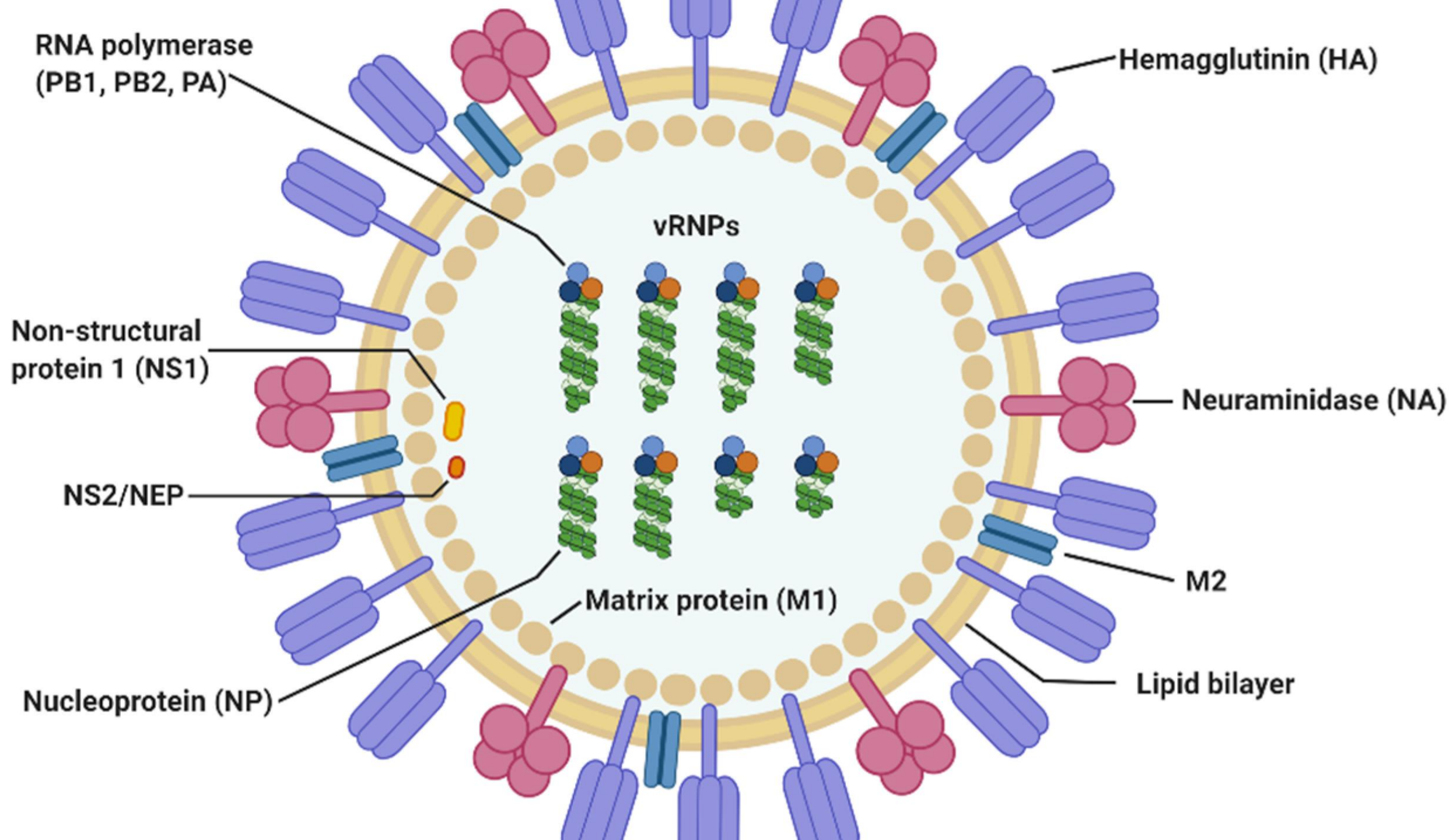
Pandemics occur when a variant of influenza A virus that contains a new hemagglutinin against which people do not have preexisting antibodies is introduced into the human population.

The pandemics caused by influenza A virus occur infrequently (the last one was in 1968), but major outbreaks caused by this virus occur virtually every year in many countries. Each year, influenza is the most common cause of respiratory tract infections that result in physician visits and hospitalizations in the United States. In the 1918 influenza pandemic, more Americans died than in World War I, World War II, the Korean War, and the Vietnam War combined. Influenza B virus does not cause pandemics, and the major outbreaks caused by this virus do not occur as often as those caused by influenza A virus. It is estimated that approximately 36,000 people die of influenza each year in the United States.

Important Properties

Influenza virus is composed of a segmented single-stranded RNA genome, a helical nucleocapsid, and an outer lipoprotein envelope. The virion contains an RNA-dependent RNA polymerase, which transcribes the negative-polarity genome into mRNA.

The envelope is covered with two different types of spikes, a hemagglutinin and a neuraminidase. Influenza A virus has 16 antigenically distinct types of hemagglutinin and 9 antigenically distinct types of neuraminidase. As discussed later, some of these types cause disease in humans, but most of the types typically cause disease in other animal species such as birds, horses, and pigs. The function of the hemagglutinin is to bind to the cell surface receptor (neuraminic acid, sialic acid) to initiate infection of the cell. In the clinical laboratory, the hemagglutinin agglutinates red blood cells, which is the basis of a diagnostic test called the hemagglutination inhibition test. The hemagglutinin is also the target of neutralizing antibody (i.e., antibody against the hemagglutinin inhibits infection of the cell). The neuraminidase cleaves neuraminic acid (sialic acid) to release progeny virus from the infected cell. The hemagglutinin functions at the beginning of infection, whereas the neuraminidase functions at the end. Neuraminidase also degrades the protective layer of mucus in the respiratory tract. This enhances the ability of the virus to gain access to the respiratory epithelial cells. Influenza viruses, especially influenza A virus, show changes in the antigenicity of their hemagglutinin and neuraminidase proteins; this property contributes to their capacity to cause devastating worldwide epidemics (pandemics).



Pathogenesis & Immunity

Influenza virus infection causes inflammation of the mucosa of upper respiratory tract sites such as the nose and pharynx, and lower respiratory tract sites such as the larynx, trachea, and bronchi. Pneumonia, which involves the alveoli may also occur.

After the virus has been inhaled, the neuraminidase degrades the protective mucus layer, allowing the virus to gain access to the cells of the upper and lower respiratory tract. The infection is limited primarily to this area because the proteases that cleave the hemagglutinin are located in the respiratory tract. Despite systemic symptoms, viremia rarely occurs. The systemic symptoms, such as severe myalgias, are due to cytokines circulating in the blood. There is necrosis of the superficial layers of the respiratory epithelium. Influenza virus pneumonia, which can complicate influenza, is interstitial in location.

Immunity depends mainly on secretory IgA in the respiratory tract. IgG is also produced but is less protective. Cytotoxic T cells also play a protective role.

Laboratory Diagnosis

Although most diagnoses of influenza are made on clinical grounds, laboratory tests are available. The test most commonly used is an enzyme-linked immunosorbent assay (ELISA) for viral antigen in respiratory secretions such as nasal or throat washings, nasal or throat swabs, or sputum. Several rapid ELISA tests suitable for a physician's office laboratory are available. Two tests (FLU OIA and QuickVue Influenza Test) are based on detection of viral antigen using monoclonal antibodies, and a third test (ZSTATFLU) is based on detection of viral neuraminidase using a substrate of the enzyme that changes color when cleaved by neuraminidase. The rationale for using the rapid tests is that treatment with the neuraminidase inhibitors should be instituted within 48 hours of the onset of symptoms. Other tests such as direct fluorescent antibody and polymerase chain reaction (PCR) are also used. Influenza can also be diagnosed by the detection of antibodies in the patient's serum.

Treatment

Oseltamivir (Tamiflu) taken orally and zanamivir (Relenza) inhaled into the nose are the two most commonly used drugs for the treatment of influenza. A third drug, perami-vir (Rapivab) is administered intravenously and became available in 2015. They are members of a class of drugs called neuraminidase inhibitors, which act by inhibiting the release of virus from infected cells. This limits the extent of the infection by reducing the spread of virus from one cell to another. These drugs are effective against both influenza A and B viruses. amantadine is effective only against influenza A, not against influenza B.

Prevention

The main mode of prevention is the vaccine, which contains both influenza A and B viruses. Prior to 2013, the vaccine was trivalent and contained recent isolates of two A strains (H1N1 and H3N2) and one B strain. In 2013, quad-valent vaccines containing two A strains and two B strains became available. The vaccine is usually reformulated each year to contain the current antigenic strains. There are two main types of influenza vaccines available in the United States, a killed vaccine and a live, attenuated vaccine. The vaccine that has been used for many years is a killed vaccine containing purified protein subunits of the virus (hemagglutinin and neuraminidase).

PARAMYXOVIRUSES

The paramyxovirus family contains four important human pathogens: measles virus, mumps virus, respiratory syncytial virus, and parainfluenza viruses. They differ from orthomyxoviruses in that their genomes are not segmented, they have a larger diameter, and their surface spikes are different. Paramyxoviruses are composed of one piece of single-stranded RNA, a helical nucleocapsid, and an outer lipoprotein envelope. The virion contains an RNA-dependent RNA polymerase, which transcribes the negative-polarity genome into mRNA. The genome is therefore not infectious. The envelope is covered with spikes, which contain hemagglutinin, neuraminidase, or a fusion protein that causes cell fusion and, in some cases, hemolysis.

MEASLES VIRUS

Disease

This virus causes measles, a disease characterized by a maculopapular rash. It occurs primarily in childhood.

Important Properties

The genome RNA and nucleocapsid of measles virus are those of a typical paramyxovirus (see earlier). The virion has two types of envelope spikes, one with hemagglutinating activity and the other with cell-fusing and hemolytic activities (see Table 39-4). It has a single serotype, and the hemagglutinin is the antigen against which neutralizing antibody is directed. Humans are the natural host.

Summary of Replicative Cycle

After adsorption to the cell surface via its hemagglutinin, the virus penetrates and uncoats and the virion RNA polymerase transcribes the negative-strand genome into mRNA. Multiple mRNAs are synthesized, each of which is translated into the specific viral proteins; no polypeptide analogous to that synthesized by poliovirus is made. The helical nucleocapsid is assembled, the matrix protein mediates the interaction with the envelope, and the virus is released by budding from the cell membrane.

Transmission & Epidemiology

Measles virus is transmitted via respiratory droplets produced by coughing and sneezing both during the prodromal period and for a few days after the rash appears.

Measles occurs worldwide, usually in outbreaks every 2 to 3 years, when the number of susceptible children reaches a high level. The WHO estimates there are 30 million cases of measles each year worldwide. In the year 2000, CDC declared measles eliminated from the United States. Elimination meant that sustained transmission within the United States no longer occurred. However, cases acquired abroad (imported cases) followed by small outbreaks continue to occur.



Measles—note
splotchy “morbilliform” maculopapular rash

Pathogenesis & Immunity

After infecting the cells lining the upper respiratory tract, the virus enters the blood and infects reticuloendothelial cells, where it replicates again. It then spreads via the blood to the skin. The rash is caused primarily by cytotoxic T cells attacking the measles virus-infected vascular endothelial cells in the skin. Antibody-mediated vasculitis may also play a role. Shortly after the rash appears, the virus can no longer be recovered and the patient can no longer spread the virus to others. Multinucleated giant cells, which form as a result of the fusion protein in the spikes, are characteristic of the lesions. Lifelong immunity occurs in individuals who have had the disease. Although IgG antibody may play a role in neutralizing the virus during the viremic stage, cell-mediated immunity is more important. The importance of cell-mediated immunity is illustrated by the fact that agammaglobulinemic children have a normal course of disease, are subsequently immune, and are protected by immunization.

Maternal antibody passes the placenta, and infants are protected during the first 6 months of life.

Infection with measles virus can transiently depress cell-mediated immunity against other intracellular micro-organisms, such as *Mycobacterium tuberculosis*, leading to a loss of purified protein derivative (PPD) skin test reactivity, reactivation of dormant organisms, and clinical disease.

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RESPIRATORY SYNCYTIAL VIRUS

Diseases

Respiratory syncytial virus (RSV) is the most important cause of pneumonia and bronchiolitis in infants. It is also an important cause of otitis media in children and of pneumonia in the elderly and in patients with chronic cardiopulmonary diseases.

Important Properties

The genome RNA and nucleocapsid are those of a typical paramyxovirus (see Table 39-1). Its surface spikes are fusion proteins, not hemagglutinins or neuraminidases (see Table 39-4). The fusion protein causes cells to fuse, forming multinucleated giant cells (syncytia), which give rise to the name of the virus. Humans are the natural hosts of RSV. For many years, RSV was thought to have one serotype; however, two sero-types, designated subgroup A and subgroup B, have been detected by monoclonal antibody tests. Antibody against the fusion protein neutralizes infectivity.

Transmission & Epidemiology

Transmission occurs via respiratory droplets and by direct contact of contaminated hands with the nose or mouth. RSV causes outbreaks of respiratory infections every win-ter, in contrast to many other "cold" viruses, which reenter the community every few years. It occurs worldwide, and virtually everyone has been infected by the age of 3 years. RSV also causes outbreaks of respiratory infections in hospitalized infants; these outbreaks can be controlled by handwashing and use of gloves, which interrupt transmission by hospital personnel.

Pathogenesis & Immunity

RSV infection in infants is more severe and more often involves the lower respiratory tract than in older children and adults. The infection is localized to the respiratory tract; viremia does not occur. The severe disease in infants may have an immuno-pathogenic mechanism. Maternal antibody passed to the infant may react with the virus, form immune complexes, and damage the respiratory tract cells. Trials with a killed vaccine resulted in more severe disease, an unexpected finding that supports such a mechanism. Most individuals have multiple infections caused by

RS, indicating that immunity is incomplete. The reason for this is unknown, but it is not due to antigenic variation of the virus. IgA respiratory antibody reduces the frequency of RSV infection as a person ages.



Laboratory Diagnosis

An enzyme immunoassay ("rapid antigen test") that detects the presence of RSV antigens in respiratory secretions is commonly used. The presence of the virus can be detected by immunofluorescence on smears of respiratory epithelium or by isolation in cell culture. The cytopathic effect in cell culture is characterized by the formation of multinucleated giant cells. A fourfold or greater rise in antibody titer is also diagnostic. A reverse transcriptase polymerase chain reaction (RT-PCR) test is also available.

Treatment

Aerosolized ribavirin (Virazole) is recommended for severely ill hospitalized infants, but there is uncertainty regarding its effectiveness. A combination of ribavirin and hyperimmune globulins against RSV may be more effective.

Prevention

There is no vaccine. Previous attempts to protect with a killed vaccine resulted in an increase in severity of symptoms. Passive immunization with a monoclonal antibody directed against the fusion protein of RSV (palivizumab, Synagis) can be used for prophylaxis in premature or immunocompromised infants. Hyperimmune globulins (RespiGam) are also available for prophylaxis in these infants and in children with chronic lung disease. Nosocomial outbreaks can be limited by handwashing and use of gloves.

PARAINFLUENZA VIRUSES

Diseases

These viruses cause croup (acute laryngotracheobronchitis), laryngitis, bronchiolitis, and pneumonia in children and a disease resembling the common cold in adults.

Important Properties

The genome RNA and nucleocapsid are those of a typical paramyxovirus (see Table 39-1). The surface spikes consist of hemagglutinin (H), neuraminidase (N), and fusion (F) proteins (see Table 39-4). The fusion protein mediates the formation of multinucleated giant cells. The H and N proteins are on the same spike; the F protein is on a separate spike. Both humans and animals are infected by parainfluenza viruses, but the animal strains do not infect humans. There are four types, which are distinguished by antigenicity, cytopathic effect, and pathogenicity (see later). Antibody to either the H or the F protein neutralizes infectivity.

Transmission & Epidemiology

These viruses are transmitted via respiratory droplets. They cause disease worldwide, primarily in the winter months.

Pathogenesis & Immunity

These viruses cause upper and lower respiratory tract disease without viremia. A large proportion of infections are subclinical. Parainfluenza viruses 1 and 2 are major causes of croup. Parainfluenza virus 3 is the most common parainfluenza virus isolated from children with lower respiratory tract infection. Parainfluenza virus 4 rarely causes disease, except for the common cold.

Laboratory Diagnosis

Most infections are diagnosed clinically. The diagnosis can be made in the laboratory either by isolating the virus in cell culture or by observing a fourfold or greater rise in antibody titer. PC assay can also be used.

Treatment & Prevention

There is neither antiviral therapy nor a vaccine available.

ADENOVIRUSES

Diseases

Adenoviruses cause a variety of upper and lower respiratory tract diseases such as pharyngitis, conjunctivitis ("pink eye"), the common cold, and pneumonia. Keratoconjunctivitis, hemorrhagic cystitis, and gastroenteritis also occur.

Important Properties

Adenoviruses are nonenveloped viruses with double-stranded linear DNA and an icosahedral nucleocapsid. They are the only viruses with a fiber protruding from each of the 12 vertices of the capsid. The fiber is the organ of attachment and is a hemagglutinin. When purified free of virions, the fiber is toxic to human cells.

Summary of Replicative Cycle

After attachment to the cell surface via its fiber, the virus penetrates and uncoats, and the viral DNA moves to the nucleus. Host cell DNA-dependent RNA polymerase transcribes the early genes, and splicing enzymes remove the RNA representing the introns, resulting in functional mRNA. (Note that introns and exons, which are common in eukaryotic DNA, were first described for adenovirus DNA.) Early mRNA is translated into nonstructural proteins in the cytoplasm. After viral DNA replication in the nucleus, late mRNA is transcribed and then translated into structural virion proteins. Viral assembly occurs in the

nucleus, and the virus is released by lysis of the cell, not by budding.

Transmission & Epidemiology

Adenoviruses are transmitted by several mechanisms: aerosol droplet, fecal-oral route, and direct inoculation of conjunctivas by tonometers or fingers. The fecal-oral route is the most common mode of transmission among young children and their families. Many species of animals are infected by strains of adenovirus, but these strains are not pathogenic for humans.

Adenovirus infections are endemic worldwide, but outbreaks occur among military recruits, apparently as a result of the close living conditions that facilitate transmission. Certain serotypes are associated with specific syndromes (e.g., types 3, 4, 7, and 21 cause respiratory disease, especially in military recruits; types 8 and 19 cause epidemic keratoconjunctivitis; types 11 and 21 cause hemorrhagic cystitis; and types 40 and 41 cause infantile gastroenteritis).

Pathogenesis & Immunity

Adenoviruses infect the mucosal epithelium of several organs (e.g., the respiratory tract [both upper and lower), the gastrointestinal tract, and the conjunctivas). Immunity based on neutralizing antibody is type-specific and

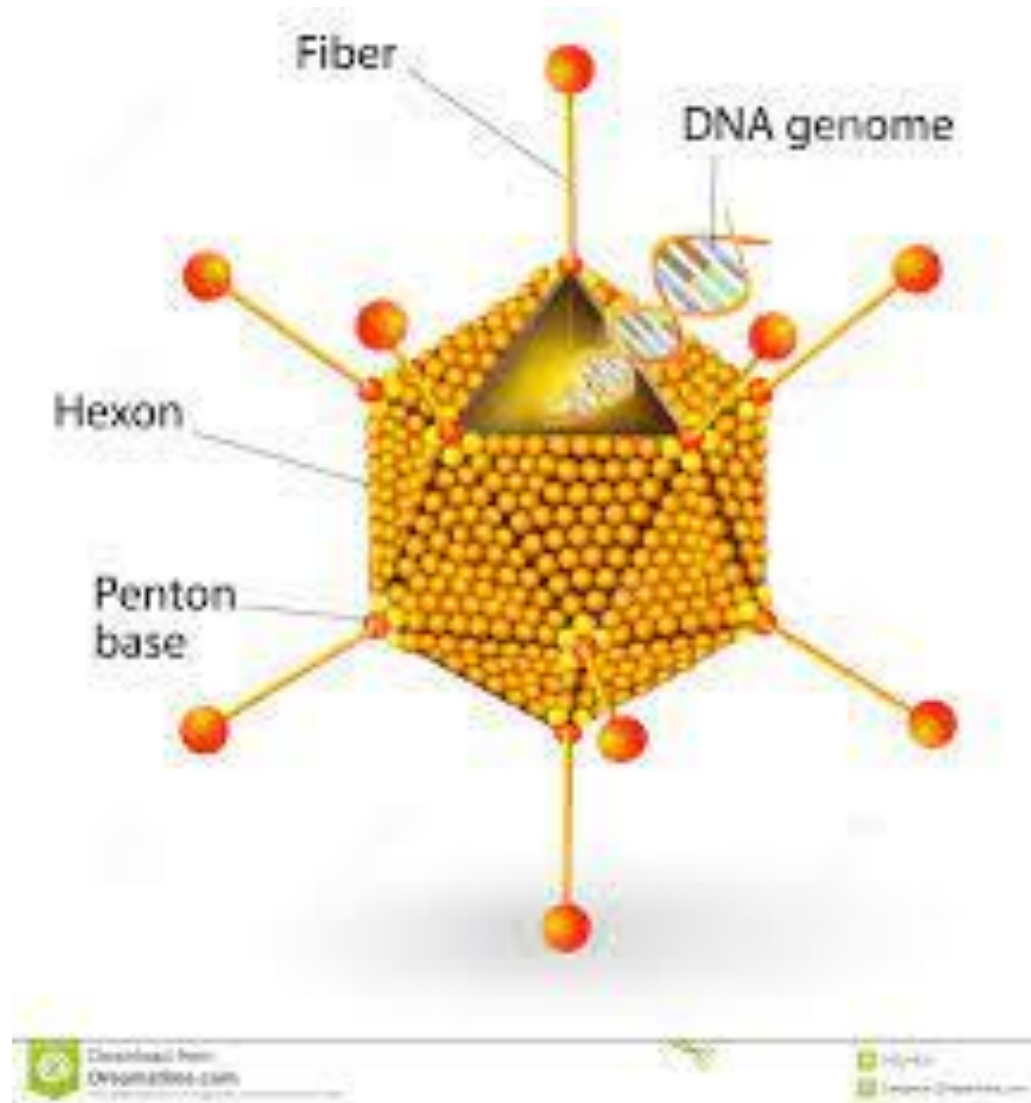
lifelong. In addition to acute infection leading to death of the cells, adenoviruses cause a latent infection, particularly in the adenoidal and tonsillar tissues of the throat. In fact, these viruses were named for the adenoids, from which they were first isolated in 1953.

Laboratory Diagnosis

The most frequent methods of diagnosis are isolation of the virus in cell culture and detection of a fourfold or greater rise in antibody titer. Complement fixation and hemagglutination inhibition are the most important serologic tests.

Treatment

There is no antiviral therapy.

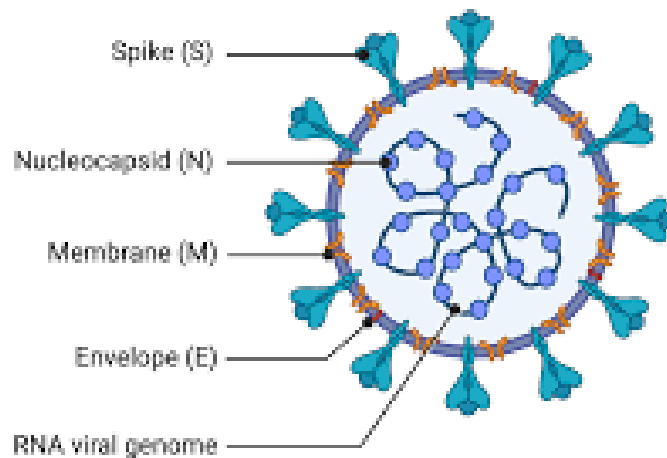


CORONAVIRUS

Diseases

Coronaviruses are an important cause of the common cold, probably second only to rhinoviruses in frequency. In 2002, a new disease, an atypical pneumonia called severe acute respiratory syndrome (SARS), emerged. In 2012, another severe pneumonia called Middle East respiratory syndrome emerged.

Coronavirus Structure



Important Properties

Coronaviruses have a nonsegmented, single-stranded, positive-polarity RNA genome. They are enveloped viruses with a helical nucleocapsid. There is no virion polymerase. In the electron microscope, prominent club-shaped spikes in the form of a corona (halo) can be seen.

There are two serotypes called 229E and OC43. The genome sequence of the coronavirus that caused the SARS (CoV-SARS) outbreak is different from that of the existing human strains. The genome sequence of different isolates of CoV-SARS is very similar, so the antigenicity of the virus is likely to be quite stable. The receptor for the SARS coronavirus on the surface of cells is angiotensin-converting enzyme-2.

Summary of Replicative Cycle

The virus adsorbs to cells via its surface spikes (hemag-glutinin), after which it enters the cytoplasm, where it is uncoated. The positive-strand genome is translated into two large polypeptides, which are self-cleaved by the virus-encoded protease. Two of these peptides aggregate to form the RNA polymerase that replicates the genome.

In addition, mRNAs are synthesized and then translated into the structural proteins. The virus is assembled and obtains its envelope from the endoplasmic reticulum, not from the plasma membrane. Replication occurs in the cytoplasm.

Transmission & Epidemiology

Coronaviruses are transmitted by the respiratory aero-sol. Infection occurs worldwide and occurs early in life, as evidenced by finding antibody in more than half of children. Outbreaks occur primarily in the winter on a 2- to 3-year cycle. SARS originated in China in November 2002 and spread rapidly to other countries. As of this writing, there have been 8300 cases and 785 deaths-a fatality rate of approximately 9%. Human-to-human transmission occurs, and some patients with SARS are thought to be "super-spread-ers." Early in the outbreak, many hospital personnel were affected, but respiratory infection control procedures have greatly reduced the spread within hospitals. There are many animal coronaviruses, and they are suspected of being the source of CoV-SARS. The horseshoe bat appears to be the natural reservoir for CoV-SARS, with the civet cat serving as an intermediate host.

Pathogenesis & Immunity

Coronavirus infection is typically limited to the mucosal cells of the respiratory tract. Approximately 50% of infections are asymptomatic, and it is unclear what role they play in the spread of infection. Immunity following infection appears to be brief, and reinfection can occur.

Pneumonia caused by SARS coronavirus is characterized by diffuse edema resulting in hypoxia. The binding of the virus to angiotensin-converting enzyme-2 (ACE-2) on the surface of respiratory tract epithelium may contribute

to the dysregulation of fluid balance that causes the edema in the alveolar space. MERS-CoV binds to CD-26 on the respiratory mucosa, not to ACE-2.

Laboratory Diagnosis

The diagnosis of the "common cold is primarily a clinical one. If SARS or MERS is suspected, antibody-based and PCR-based tests can be used.

Treatment & Prevention

There is no antiviral therapy or vaccine available. A combination of ribavirin and steroids has been tried in the treatment of life-threatening cases of SARS, but their efficacy is uncertain.

POXVIRUSES

The poxvirus family includes three viruses of medical importance: smallpox virus, vaccinia virus, and molluscum contagiosum virus. Poxviruses are the largest and most complex viruses.

SMALLPOX VIRUS

Disease

Smallpox virus, also called variola virus, is the agent of smallpox, the only human disease that has been eradicated from the face of the Earth. Eradication was achieved by the widespread use of the smallpox vaccine. There is concern regarding the use of smallpox virus as an agent of bioterror-ism. . (Note that rinderpest, a disease primarily of cattle, has also been eradicated by using the vaccine against rinderpest virus (RPV). RPV is a paramyxovirus related to measles virus.)

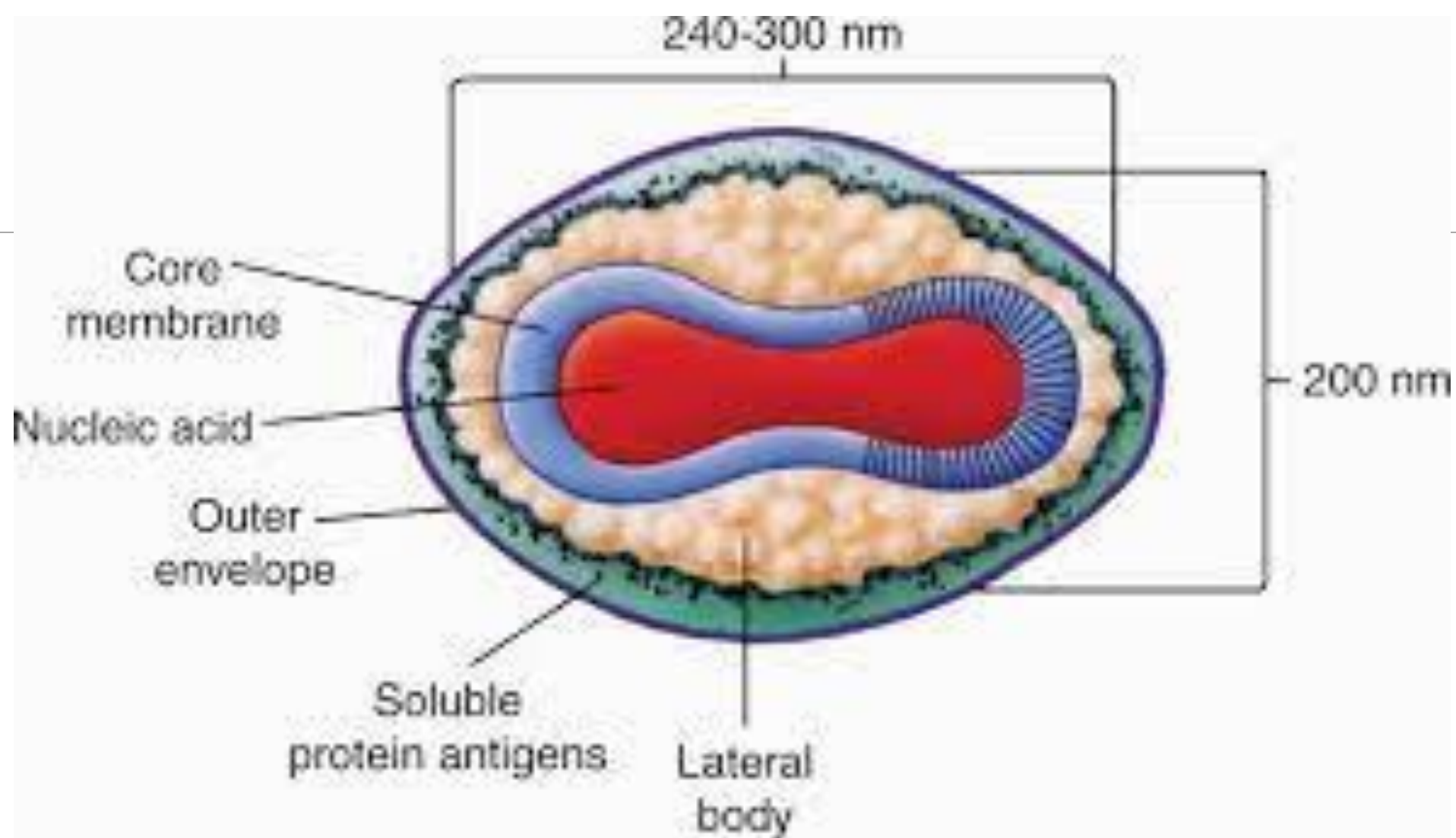
Important Properties

Poxviruses are brick-shaped particles containing linear double-stranded DNA, a disk-shaped core within a double membrane, and a lipoprotein envelope. The virion contains a DNA-dependent RNA polymerase. This enzyme is required because the virus replicates in the cytoplasm and does not have access to the cellular RNA polymerase, which is located in the nucleus.

Smallpox virus has a single, stable serotype, which is the key to the success of the vaccine. If the antigenicity varied as it does in influenza virus, eradication would not have succeeded. Smallpox virus infects only humans; there is no animal reservoir.

Summary of Replicative Cycle

The following description of the replicative cycle is based on studies with vaccinia virus, as it is much less likely to cause human disease than smallpox virus. After penetration of the cell and uncoating, the virion DNA-dependent RNA polymerase synthesizes early mRNA, which is translated into early, nonstructural proteins, mainly enzymes required for subsequent steps in viral replication. The viral DNA then is replicated, after which late, structural proteins are synthesized that will form the progeny virions. The virions are assembled and acquire their envelopes by budding from the cell membrane as they are released from the cell. Note that all steps in replication occur in the cytoplasm, which is unusual for a DNA virus.



Transmission & Epidemiology

Smallpox virus is transmitted via respiratory aerosol or by direct contact with virus either in the skin lesions or on fomites such as bedding.

Pathogenesis & Immunity

Smallpox begins when the virus infects the upper respiratory tract and local lymph nodes and then enters the blood (primary viremia). Internal organs are infected; then the virus reenters the blood (secondary viremia) and spreads to the skin. These events occur during the incubation period, when the patient is still well. The rash is the result of virus replication in the skin, followed by damage caused by cytotoxic T cells attacking virus-infected cells. Immunity following smallpox disease is lifelong; immunity following vaccination lasts about 10 years.

Laboratory Diagnosis

In the past when the disease occurred, the diagnosis was made either by growing the virus in cell culture or chick embryos or by detecting viral antigens in vesicular fluid by immunofluorescence.

Prevention

The disease was eradicated by global use of the vaccine, which contains live, attenuated vaccinia virus. The success of the vaccine is dependent on five critical factors: (1) smallpox virus has a single, stable serotype; (2) there is no animal reservoir, and humans are the only hosts; (3) the antibody response is prompt, and therefore exposed persons can be protected; (4) the disease is easily recognized clinically, and therefore exposed persons can be immunized promptly; and (5) there is no carrier state or subclinical infection.