Introduction to viruses

What are viruses?

Well… some textbooks describe a virus as "a bad news wrapped up in a protein coat", the *bad news* being the nucleic acid either DNA or RNA but not both. In short, viruses are obligate intracellular parasites. This means that they can't live or replicate outside a living cell. To replicate, they need the machinery of a host cell such as (lysozymes, mitochondria, ribosomes… etc.) for protein synthesis and other purposes.

Structure of a virus:

Knowing the structure of viruses is fundamental in understanding and classification of viruses. The structure of a virus depends on its size. If it is small then it has a simple structure, if it is big (such as Pox) then it has a complex structure.

- **Nucleic acid genome**: that is either DNA or RNA but never both.
- **Protein coat (capsid)**: for protection from rough environments.
- **Lipid envelope**: found in some viruses. It is derived from host cell plasma membrane
- **Size**: they are very small (20-400 nm)

Basics:

- **Different Structure**: viruses have a different structure from all other microorganisms in that other microorganisms have two types of nucleic acids (both DNA & RNA) together while viruses have only one. In this aspect we can divide viruses into two main groups: DNA viruses and RNA viruses. Even within one group of viruses (e.g. DNA viruses) we have different structures (size, shape, type of the capsid, number of capsomeres… etc.).

- **Different methods of replication**: the method of replication greatly depends on the type of nucleic acid that the virus contains. DNA viruses do not replicate like RNA viruses, especially on a molecular level. Even within the same family of nucleic acid viruses we have different methods of replication depending on whether the nucleic acid is single-stranded or double-stranded (in case of RNA). If it is single-stranded, it could have a positive sense or a negative sense or it could be either linear or circular.

- The reason we study the structure and different replication methods of viruses is implicated on **diagnosis**, **treatment** and **prevention**. If we know the structure of the virus, we have a proper diagnosis (i.e. we know our enemy). Then we can treat it accordingly if we have a treatment. If we don't have a treatment we can apply a proper strategy for prevention (e.g. vaccination).

Control Methods:

To control a viral disease we have to investigate several points:

1) Reservoir: the source that the virus comes from. E.g. humans, migrating birds, domestic animals.

2) Mode of Transmission: the mode in which the virus is transmitted from the reservoir. If we know the means, we can eliminate the mode of transmission. Examples:

- Direct contact
- Insects (mosquitos, ticks, and fleas): they suck blood from an infected animal and deposit the virus inside humans with their saliva as they bite.
- Animals

3) Methods of Inactivation: we have to find antiseptics to inactivate viruses to clean community hospitals. E.g. alcohol, potassium hypochlorite, iodine and others.

4) Vaccines: this is the most important point.

5) Antiviral drugs

6) Development of drug resistance by the virus

Emerging Viral Diseases:

- **Emerging**: happening for the first time (a new viral disease). E.g. AIDS, HPS, Monkey Pox, WNV, SARS.
- **Re-emerging:** an existing virus that emerges in a new strain. E.g. influenza → swine flu

Consequences of viral infections:

It has been reported that 50% of absenteeism in all fields of work is caused by a viral infections especially in children because they are more prone to get a viral infection as they have an underdeveloped immune system. The consequences of viral infections depend on the pathogenicity of the virus.

1) Suffering for a couple of days **followed by complete recovery** as in case of a rhino virus (low pathogenicity)

2) Persistent disease: a disease that will be with you for the rest of your life. E.g. Hepatits B.

3) Fatal disease: rabies, hemorrhagic fever, yellow fever, hepatitis B and C if not diagnosed early.

4) Congenital disease: diseases transmitted from a pregnant mother to her fetus. E.g. rubella, cytomegalovirus, HIV.

5) Contributory factor in cancer (oncogenic viruses): viral infections that predispose to cancer.
E.g. Leukemia, HTLV 1 and 2 (a retrovirus), Herpes (some of them).

6) Contributory factor in other diseases: influenza →secondary bacterial infection. That is why in case of some viral infections we prescribe antibiotics to prevent secondary bacterial infections (complication).

7) Some are asymptomatic: these are subclinical infections in which the patient does not exhibit any signs or symptoms and acts as a carrier for the virus. E.g. Hepatits A.

Uses of viruses:

1) Vaccines

2) Gene Therapy: this can be used to cure certain genetic diseases (e.g. single-gene defects), where the gene is delivered to the body of the patient by injecting him with a harmless virus (e.g. adenovirus) containing the gene. Then the virus will reproduce inside the patient's body replacing the deficiency of the gene.

3) Host Cell Investigation

Paramyxoviridae family

They are large, enveloped RNA viruses (single stranded, helical, negative sense) of 150-350nm in diameter.

Difference from orthomyxoviridae family

1. Their nucleic acid is non-segmented, unlike influenza which has 8 segments
2. Influenza has two glycoproteins (spikes): **hemagglutinin* **neuraminidase.* Paramyxoviridae also have two spikes but the HA and NA are located on a single spike, while the other spike is **fusion F protein** used for fusion.

- All of us have been infected with a paramyxovirus during childhood. So all children by age 5 or 6 have antibody against one of the paramyxoviruses since they have a worldwide distribution.

This family is divided into **two** subfamilies:

Paramyxovirinae	Pneumovirinae
• **Paramyxovirus** (now called Respirovirus) ➢ Parainfluenza type 1 & 3 ➢ Sendai virus (in mice) • **Rubulavirus** ➢ Parainfluenza type 2 & 4 ➢ Mumps • **Morbillivirus** ➢ Measles ➢ Canine distemper virus (in dogs)	• **Pneumovirus** ➢ **Respiratory Syncytial Virus**: it is found in our community; it contains G-protein but it doesn't have hemagglutinin and neuraminidase (this is a difference between the pneumovirinae and paramyxovirinae ***subfamilies***) • **Human metapneumovirus** ➢ before 10 years there was only animal metapneumovirus but 10 or 11 years ago they isolated metapneumovirus in children with acute bronchiolitis and pneumonia in Holand

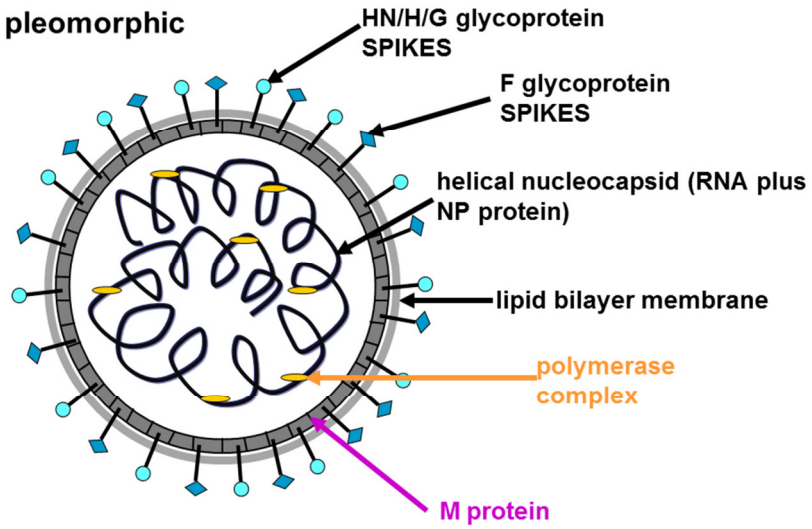
GENERAL PROPERTIES OF PARAMYXOVIRIDAE FAMILY

- 150 – 350nm in diameter
- Spherical or pleomorphic in shape
- Enveloped
- Single stranded, negative sense, unsegmented, helical RNA nucleic acid; the RNA is associated with three important proteins (nucleoprotein, phosphoprotein, large protein). The proteins are associated with polymerase complex enzyme which is actually linked to nucleoprotein of the virus. These proteins are important for differentiation between the viruses
- Like influenza they have a lipid bilayer membrane which is associated with virus specific glycoprotein (**HA & NA** on one spike and **F protein** on another)
- Fusion protein promotes fusion of the viral envelope with the cell membrane of host cells and formation of syncytia (multinucleated giant cells)
- They have the property of hemagglutination (inhibition of RBCs)

Other properties:

- All members are labile (sensitive to environmental factors), but can survive on surfaces for a few hours (6 – 10 hours)
- They are highly infectious by direct contact, contaminated fomite, sneezing and coughing
- They are susceptible to destruction by soaps
- They are hemadsorbing viruses since they have HA
- They are antigenically stable (in contrast to influenza) since they are *unsegmented*

Diagrammatic illustration of paramyxovirus



M-protein (matrix protein): is very important for maturation of the virus. It is a hydrophobic protein located inside the virus to give the shape of the virus, they are important in assembly of the virus in the cytoplasm of the infected cells.

PATHOGENESIS (similar to influenza)

- We get the virus by inhalation
- It gets into our system through the *nasopharyngeal* mucosa, then it will spread to the lower respiratory tract within 1-3 days
- Replication occurs in the *cytoplasm*
- RNA polymerase is very important for translation of mRNA

EPIDEMIOLOGY

- They are an important cause of respiratory disease
- Most people are infected by age 5
- They cause *epidemic* and *sporadic* infections
- **Seasonality**: fall and early winter
- Type **1** & **2** cause epidemics in fall and early winter
- Type **3** & **4** cause some sort of sporadic infection
- Stable on surfaces (up to 10 hours)
- Transmitted by large droplets during sneezing
- 90% of individuals are *asymptomatic* but they shed the virus by sneezing
- Shedding lasts 1 week after infection
- Infection is more severe in immunocompromised patients

ICEBERGE PHENOMENA

- Only small percentage show classical disease presentation
- Majority are asymptomatic but highly infectious

CLINICAL FEATURES

- Incubation period: short (6 days)
- Primary infection or reinfection may occur (due to presence of different types and subtypes)
- Reinfection is usually milder than the primary infection
- Fever
- Upper and Lower Respiratory infection characterized by:
  - **CROUP**: Acute laryngeotracheobronchitis specially in children under 2 years then they will get allergic bronchitis due to formation of high titers of IgE
  - Narrowing of air passage due to inflammation of epithelial cells of upper respiratory tract
  - Bronchiolitis
  - Pneumonia
- Seasonal outbreak in fall & winter (mainly parainfluenza type 1 & 2)
- Type 3 & 4 cause milder, sporadic infections

TREATMENT

- Symptomatic treatment
- Humidification
- No antiviral agent or chemotherapy
- No vaccine

Type 3	Type 4
• Primary infection occurs in children under 5 years of age • Less common cause of bronchiolitis & pneumonia than RSV [RSV is prime cause of bronchiolitis & pneumonia in infants under 4 years of age] • Less common cause of CROP than type 1 & 2 • Causes sporadic infection in non-winter seasons	• Even the primary infection is milder than that of type 1 & 2 • It causes upper respiratory tract infection more than lower • Two subtypes 4A & 4B according to protein associated with nucleocapsid. Some of them are differentiated by G-protein or attachment protein localized on the envelope of the virus.

DIAGNOSIS

- Signs and symptoms of all strains are similar
- Antigenic detection from nasopharyngeal secretion or swab. We look for Ag in epithelial cells sloughed by the nasopharyngeal swab by ELISA or direct fluorescent technique.
- **Virological Transport media:** are used for transporting the virus to the lab while preventing desiccation of the virus.
- Tissue culture (it takes weeks)
- **Serology**: we take two samples. The **first** is taken during appearance of the symptoms and the **second** is taken *two weeks* after the first one. Then we run the samples by ELISA or HA inhibition technique to demonstrate 4-fold difference

PREVENTION

- Personal hygiene
- Hand washing
- Sanitizer gels
- Prevention of surface contamination
- Avoidance of inoculation of eye and mouth

Respiratory syncytial virus

Introduction

It is a negative-sense, single-stranded RNA virus of the family *Paramyxoviridae*, genus *Pneumovirus*. It is 100 - 350 nm, spherical or pleomorphic (like parainfluenza). Its name comes from the fact that F proteins on the surface of the virus cause the cell membranes on nearby cells to merge, forming **syncytia** (multinucleated giant cells in the lung; they can be detected by direct fluorescent technique).

This virus has been isolated in 1956 from monkeys, chimpanzees and also human beings who got the infection from chimpanzees. So the virus has been adapted to live in the human body and cause infection.

There are two subtypes of respiratory syncytial virus: **A** & **B** which are differentiated based on their G-proteins.

There are some structural and non-structural proteins. We have F (Fusion) and G (Attachment) protein but we don't have hemagglutinin and neuraminidase proteins. So this virus cannot hemadsorb or hemagglutinate RBCs.

Pathogenesis

- It is a respiratory virus
- Entry from mucosa of the nose and sometimes the eyes
- Cell to cell spread with syncytial formation
- Mucosal edema
- Increased mucin secretion
- Cell necrosis and sloughing leading to obstruction of the lumina by mucin and cell debris
- There is peribronchial lymphocyte infiltration
- Recovery from the infection is usually due to cell-mediated immunity.

Epidemiology

- The distribution of the virus is worldwide. It is also endemic in our community, especially in winter
- 90% of children under the age of four or five have been infected with RSV
- Incubation period is about 2-8 days
- The virus can survive on contaminated surfaces up to 6 hours
- Transmission by droplets, fomites, sneezing, coughing
- Nosocomial infection is common
- There is asymptomatic viral shedding
- Viral shedding lasts 1-3 weeks

Types of infection

- **Upper respiratory infection (mild):** Fever, rhinitis, pharyngitis
- **Lower respiratory infection (severe):** bronchiolitis and pneumonia, lethargy, coughing → usually seen in immunocompromised patients

Lab diagnosis

- We take a nasal swab or tracheobronchial secretion (aspirate) by special tubes
- We must process the virus immediately to prevent desiccation
- Positive results are confirmed by ELISA or direct immunofluorescent technique

Treatment

Usually supportive (fluids, oxygen, respiratory support), but if the case is fatal we can use Ribavirin which is a synthetic guanosine analogue... it is given by spray

Prevention

- Hand-washing, wearing gloves and masks
- Isolation of the patients
- Immunization (was done by a formalin-inactivated vaccine but now it is stopped due to high side effects)

Human metapneumovirus

- It is a negative-sense single-stranded RNA virus of the sub-family *Pneumovirinae*, genus *Metapneumovirus*.
- It has been recently isolated (10 or 11 years ago) in Holand in children under 4 or 5 years of age. It is also endemic in our community.
- It causes bronchiolitis and pneumonia in children
- There are two types: **A** & **B**
- It is usually seen as a co-infection with RSV
- It causes upper and lower respiratory infection
- It can cause otitis media, bronchiolitis and pneumonia

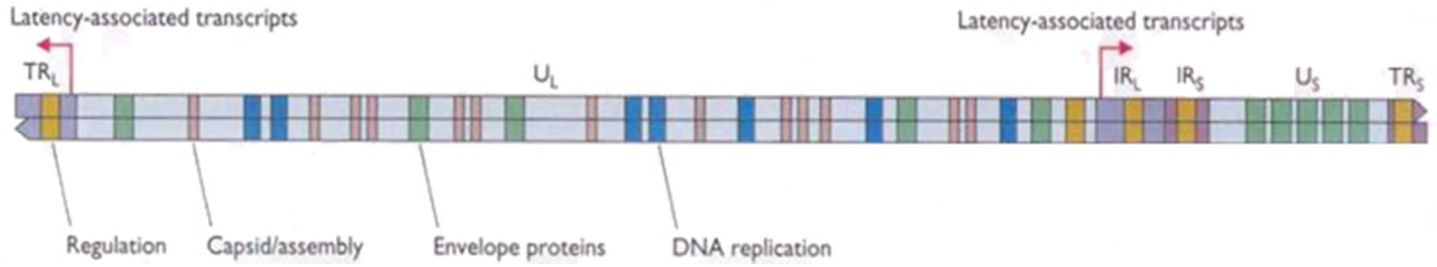
Herpes viruses

Introduction

The Herpetoviridae family is a complicated family of viruses. In this family we have 25 different viruses which infect both humans and different species of animals. Only 8 of the viruses are known to cause infections in humans. Each one causes different clinical manifestations which can be seen on a daily basis in hospitals.

Structure

Herpesviruses all share a common structure- all are enveloped and have a relatively large double-stranded, linear DNA genome. They are complicated because they contain so many different genes that control different functions of the virus. If you look at the genomic structure of the DNA of the herpes simplex virus, you can see different genes controlling different functions of the virus from Regulation to Transcription to Translation to Maturation and so on. These groups of viruses have the property of invading and replicating in nervous cells, while no other virus can invade the CNS and replicate to cause pathology in the CNS.



After replication in the CNS these viruses will go dormant and cause a latent infection due to the presence of latency associated transcripts. It means that during the replication cycle of these viruses in the CNS, they produce a transcript (i.e. the latency transcript) for the viruses to become latent in the CNS (it will sleep in the CNS) for a while and then reactivated when the immune system is suppressed. The human herpes viruses are the following:

Viruses of humans	Common name	Subfamily
Human herpes virus 1	Herpes simplex type 1	Alpha
Human herpes virus 2	Herpes simplex type 2	Alpha
Human herpes virus 3	Varicella-zoster	Alpha
Human herpes virus 4	Epstein-Barr	Gamma
Human herpes virus 5	Cytomegalovirus	Beta
Human herpes virus 6/7	exanthum subitum roseola infantum	Beta
Human herpes virus 8	Kaposi's Sarcoma-associated	Gamma

These three stages produce alpha, beta and gamma proteins. These proteins (especially alpha and beta) are acting as enzymes to proceed with the replication cycle.

- **Immediate early transcription** produces alpha proteins
- **Delayed early transcription** produces beta proteins
- **Late stage transcription** is concerned with development, maturation and release of the virus from the infected cells.

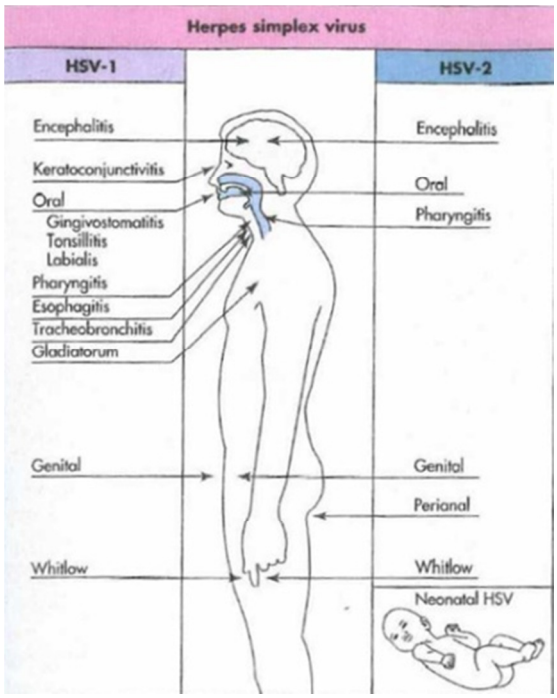
Subfamilies

- *Three* of the viruses (Herpes simplex type 1 & 2 and Varicella-zoster) are in the **alpha** subfamily and usually infect epithelial cells.
- **Cytomegalovirus** (along with human herpesvirus 6/7) belongs to the **beta** subfamily; **CMV** is an important virus which can cause congenital abnormalities.
- **Epstein - Barr virus** (along with human herpesvirus 8) belongs to **gamma** subfamily; **EBV** has been isolated from AIDS patients who had Kaposi's sarcoma.

We can distinguish these viruses according to their target cells, site of latency (usually neurons) and means of spread

Subfamily	Virus	Primary target cell	Site of latency	Means of spread
Alpha-Herpesvirinae				
Human herpesvirus 1	Herpes simplex type 1 (HSV-1)	Mucoepithelial	Neuron	Close contact
Human herpesvirus 2	Herpes simplex type 2 (HSV-2)	Mucoepithelial	Neuron	Close contact
Human herpesvirus 3	Varicella zoster virus (VZV)	Mucoepithelial	Neuron	Respiratory & close contact
Beta-Herpesvirinae				
Human herpesvirus 5	Cytomegalovirus (CMV)	Monocyte, lymphocyte, and epithelial cells	Monocyte, lymphocyte, and ?	Close contact, transfusions, tissue transplant, and congenital
Human herpesvirus 6	Herpes lymphotropic virus (HHV6)	T lymphocytes and ?	T lymphocytes and ?	Respiratory and close contact ?
Human herpesvirus 7	Human herpesvirus 7 (HHV7)	T lymphocytes and ?	T lymphocytes and ?	?
Gamma-Herpesvirinae				
Human herpesvirus 4	Epstein-Barr virus (EBV)	B lymphocyte and epithelial cells	B lymphocyte	Close contact (kissing disease)
? indicates that other cells may also be the primary target or site of latency.				

Herpes simplex **type 1** usually causes infections from the waist-line and above (Encephalitis, Keratoconjuctivitis, Gingivostomatitis, Tonsilitis, Labialis…etc) while **type 2** causes infections from waist-line and below (genital, perianal and neonatal herpes). Sometimes Herpes Simplex type 1 may cause genital infections and *whitlow* (infection of the fingers most commonly contracted by dental workers and medical workers exposed to oral secretions).



Immune Response to Herpes Infections

- **Interferon** is the first line of defense. Then we have the humoral antibody (the neutralizing antibody). Then we have complement-mediated lysis of infected cells. The neutralizing antibodies can be secreted with the milk so breastfeeding is very important for infants.
- **Cell-Mediated (T-lymphocyte) immunity** is the most important arm of immunity against herpes viruses and even if there is a defect in humoral immunity such as in agammaglobulinemics, they can recover from infection if they have normal T cell functions.
- Apart from the interferon we have the cytotoxic T-lymphocytes, natural killer cells, macrophages…etc

Neurovirulence and Latency in HSV and VZV

Herpes simplex and varicella zoster viruses have two unique biologic properties, which is the capacity to invade and replicate in the CNS.

- Virus lies dormant (*latent infection*) for a period of time but may become reactivated (*due to immune suppression*) even in the presence of neutralizing antibodies because they are not as effective as T-lymphocytes.
- Most studies indicate that the **sensory ganglia** are the source of virus that produces recurrent skin lesions-**trigeminal** ganglia in **type 1** and **sacral** ganglia in **type 2** and some of the dorsal ganglia for the varicella-zoster virus

Neurolatency hypotheses

- **Dynamic state:** persistence of low levels of infectious virus in sensory ganglia, neurons may or may not be killed. This leads to retrograde vs anterograde transport of virus through peripheral sensory nerve endings.
- **Static state:** DNA of virus is maintained in a non-replicating state at some extrachromosomal site in neuron or is integrated into cellular DNA.

Chemotherapy

- **HSV and VZV:** acyclovir (*aka* Cyclovir, Zovirax) is effective because these viruses contain thymidine kinase enzyme.
- **CMV:** ganciclovir; phosphonoacetate, interferon
- **NOTE:** the chemotherapy can't destroy the dormant stage of the viruses

Agents that inactivate the viruses

- Since the viruses have a lipid enveloped, agents such as ethyl ether, chloroform, glutaraldehyde can inactivate the virus by dissolving the envelope.

Immunologic approaches

- There is no vaccination for herpes simplex type 1 & 2, but we have a vaccine for Varicella-zoster (chicken pox) which is given with MMR (*called MMRV*) in US and Europe.

Diseases

The viruses cause a **primary** infection, then they become dormant, after that they are reactivated to cause a **recurrent** infection.

Virus	Pathogenesis	Symptoms	Target Group
HSV-1	Primary infection	Pharyngitis, Tonsillitis, Gingivostomatitis Eczema herpeticum Keratoconjuctivitis	Children & young adults Atopics Young adults
HSV-2	Primary infection	Vulvovaginitis, Herpes progenitalist Neonatal infections	Children & young adults Newborns
HSV-1	Recurrent infection	Herpes labialis, Keratoconjuctivitis, Encephalitis	Children & young adults
HSV-2	Recurrent infection	Herpes genitalis, Meningoencephalitis	Children & young adults

- **HSV isolated from lesions occurring above the waistline is usually type 1 and HSV isolated from sites below the waistline is usually type 2.** Nonetheless, because of oral-genital contact, either type of virus may be found in either location.
- **Primary HSV-1 infections are most common in childhood.** The usual manifestation in young children is gingivostomatitis; in adolescents it is tonsillitis or pharyngitis.
- **The prevalence of genital herpes bears a strong relationship to sexual promiscuity.** Genital HSV-2 infections are on the increase and are now second in frequency only to gonorrhea.

HSV Diseases:

Primary Infections:

1) **Gingivostomatitis (caused by type 1)**: affects children 1-6 years old. No seasonal distribution. Accompanied by fever, sore mouth and lymphadenopathy.
2) **Vulvovaginitis (type 2)**: involves mucous membranes and skin of the labia and lower vagina. Ulcers are accompanied by fever and regional lymphadenopathy. In males: herpes progenetialis.
3) **Meningoencephalitis (type 1, 2):** may result from a primary infection of the CNS; however, in some instances, illness follows viral **recurrence** in individuals with pre-existing antibodies.
4) **Keratoconjunctivitis (type 1)**: corneal ulcerations induced by herpesvirus may be quite deep and can result in blindness.
5) **Eczema herpeticum (type 1)**: complication of eczema or sever atopic dermatitis. The abraded weeping and denuded skin is inoculated with virus which spreads widely in the absence of the protective cornified epithelium.
6) **Burn injury:** involvement of abraded and injured skin -- may be quite severe.
7) **Neonatal disease (type 2):** disseminated, visceral and congenital infections…fetus is at risk when mother is shedding virus in birth canal.

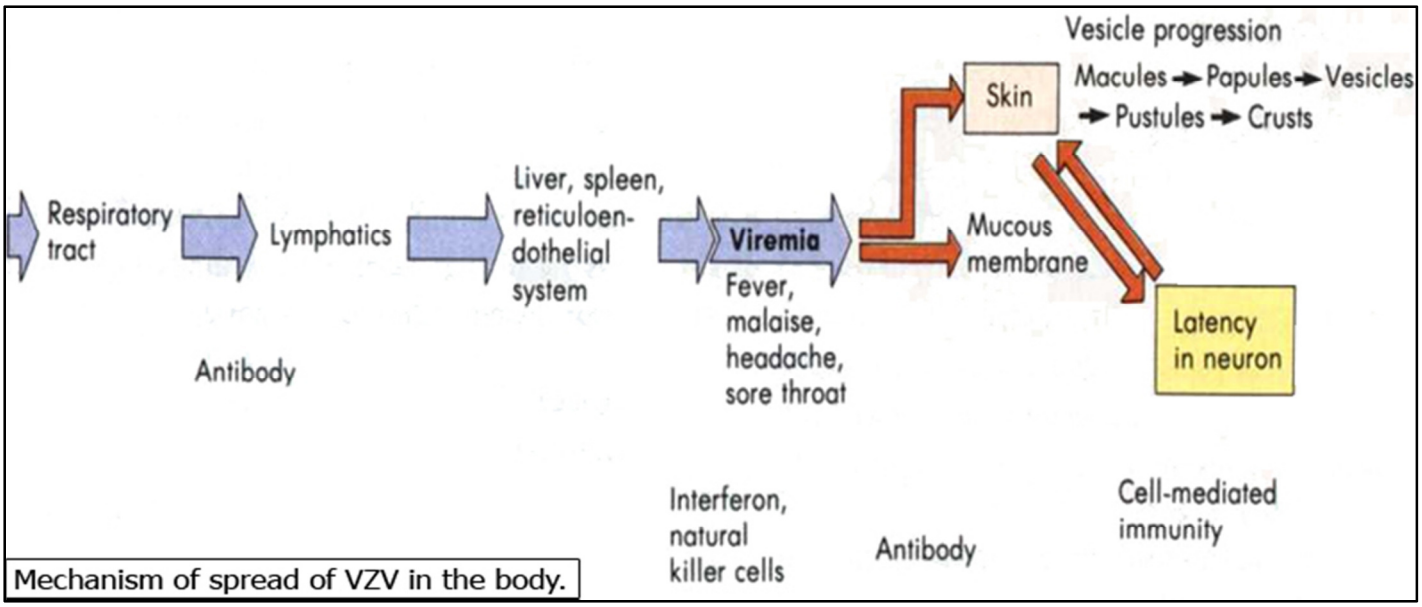
Recurrent Infections:

- Cold sores (herpes labialis)
- Genital lesions (herpes genitalis)
- Keratoconjunctivitis
- Encephalitis
- Meningoencephalitis

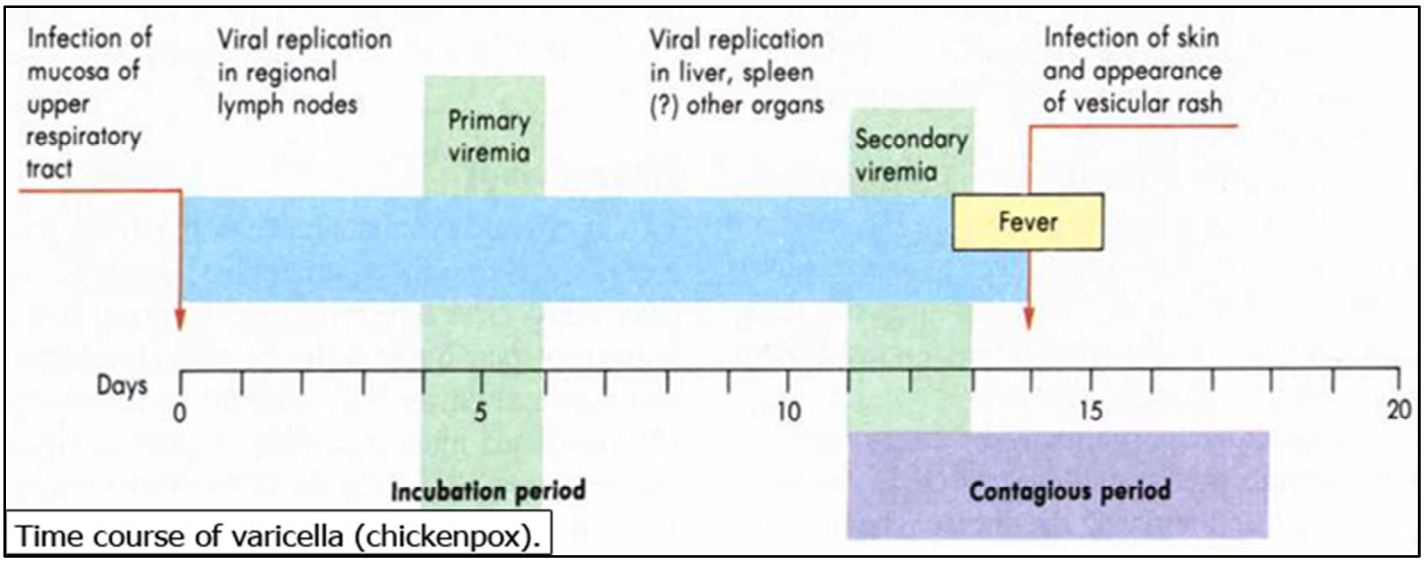
Other Herpes Virus Diseases:

1) **Varicella** (chickenpox): Usually mild disease (except in immunosuppressed) with characteristic rash and pox formation. Begins as respiratory infection then disseminates.
2) **Herpes Zoster** (shingles): Reactivation of latent varicella virus… Formation of painful vesicles unilaterally on the dermatome supplied by the dorsal ganglia…cause of reactivation is immune suppression.
3) **Cytomegaloviruses** (CMV): In adults it causes a mild or subclinical infection. In pregnant women it causes congenital infection like Rubella virus. It is also responsible for a form of mononucleosis which is heterophile **negative**.
4) **Epstein-Barr virus** (EBV): Etiologic agent of infectious mononucleosis (primary target is B-cells) which is heterophile **positive**.

NOTE: Varicella-Zoster is a single virus named by two scientists and it causes both Chickenpox (Varicella) and Shingles (Zoster).



Mechanism of spread of VZV in the body.

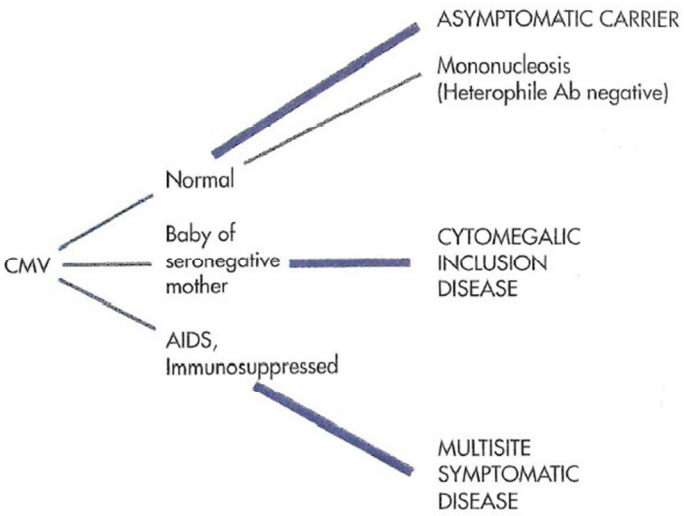


Time course of varicella (chickenpox).

FDA –approved antiviral treatments for herpesvirus infections	
Herpes simplex 1 and 2	**Epstein-Barr virus**
Acyclovir	None
Penciclovir	
Valacyclovir	
Famciclovir	
Adenosine arabinoside	
Iododeoxyuridine	
Trifluridine	
Varicella-Zoster Virus	**Cytomegalovirus**
Acyclovir	Ganciclovir
Famciclovir	Valganciclovir
Valacyclovir	Foscarnet
Varicella-zoster immune globulin	Cidofovir
Zoster immune plasma	
Live vaccine	

The antivirals used for Herpes simplex type 1 & 2 and Varicella-Zoster are not used for Cytomegalovirus because these viruses (HSV-1 & 2 and VZV) contain thymidine kinase enzyme which converts the drugs to their active forms by phosphorylastion while CMV doesn't have this enzyme.

Cytomegalovirus disease



- If it is acquired by direct contact for the first time (primary infection) it is asymptomatic or causes mononucleosis-like disease.
- If it acquired by blood transfusion (post-transfusion syndrome): fever and mononucleosis, FUO (Fever of Unknown Origin), granulomatous hepatitis.
- If a pregnant woman gets this virus during the first trimester this will cause congenital infection (called cytomegalic inclusion disease) which will cause formation of giant cells in the infected organ (*CYTO MEGAL*= giant cell). The clinical picture is similar to Rubella virus infection: petechial rash, hepatosplenomegaly, thrombocytopenia, anemia, CNS damage, low birth weight, mental retardation, partial deafness and eye disease. This disease belongs to the **TORCH** syndrome which stands for **To**xoplasma, **R**ubella, **C**ytomegalovirus and **H**erpes simplex virus. In developed countries there is a test for **TORCH** before marriage to prevent congenital abnormalities.
- If the patient has T-cell impairment, the viral infection will be severe because cellular immunity is an important arm in protection and recovery from viral infections.

CMV Routes of Infection

- Saliva
- Sexual contact
- Transfusion
- Organ transplant (usually renal)

CMV Diagnosis

It is difficult to diagnose CMV depending on clinical data alone. We have to do viral isolation by taking a specimen and inoculating it into a tissue culture in the lab so that we can get a cytopathic effect in a week or two. We usually use the human fibroblastic cells as a tissue culture (spindle-shaped cells). When they become infected with CMV they become rounded (ballooned). We can also use serology by indirect immunofluorescence, cytology on concentrated urine.

CMV Treatment

In AIDS patients we have to treat the disease because in immunocompromised patients the infection may be very severe. We may have retinitis, pneumonitis, and hepatitis. We can use *cidofovir and ganciclovir.* Until now there is no vaccine against this virus.

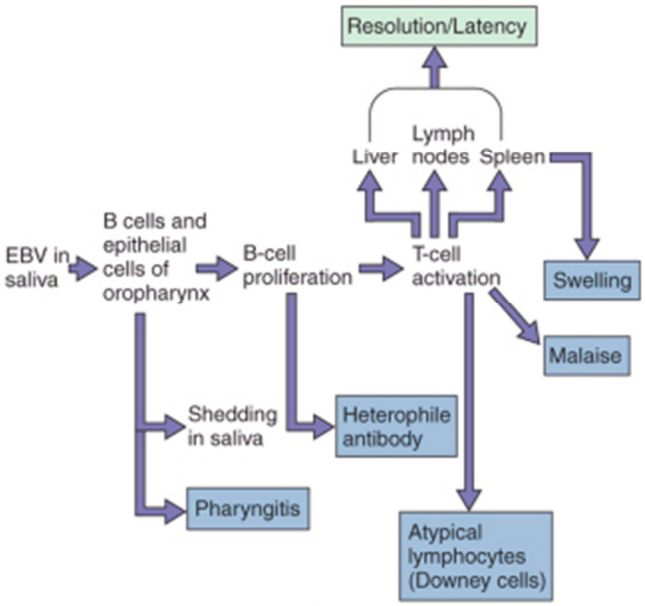
Epstein - Barr virus

- EBV is a herpesvirus first seen by electron microscopy of neoplastic cells from a Burkitt's lymphoma (BL) in 1964.
- Antibody to EBV is found in sera of almost all patients with BL or nasopharyngeal carcinoma.
- Antibody to EBV was found in 80% of low-income adults in Philadelphia and 100% of adults in developing countries; most didn't have BL.
- EBV usually results in infectious mononucleosis (IM) in North America and BL in Africa and other parts of the world. It is also associated with nasopharyngeal carcinoma.

EBV Disease Characteristics

- EBV can be cultured in normal human B lymphocytes (virus attaches to complement C3b receptor) which then begin to behave like neoplastic lymphoma cells. These cells undergo blastogenesis and begin secreting vast amounts of polyclonal (heterophile) immunoglobulins.
- Infection is followed by the prompt appearance of cytotoxic T cells which proceed to turn off activated B cells (the "atypical lymphocytes" are the T cells).
- ***Paul-Bunnell test:*** Heterophil Ab (IgM antibodies which agglutinate sheep and horse RBCs) appears in 80% of patients with Infectious Mononucleosis. These antibodies appear approximately four weeks after onset of symptoms, but are not EBV specific antibodies.

Pathogenesis of EBV



Diagnostic Procedures For Herpesviruses

1) **Cell Culture:** Cell rounding > fusion > giant cell formation (HSV)
2) **Immunofluorescence**: Intense nuclear staining for all herpesviruses
3) **ELISA and Western Blot:** Specificity based on glycoprotein
4) **PCR**
5) **TORCH:** Serologic assay

Picornaviridae family

They are single-stranded, positive-sense, RNA viruses. This family has 9 different genera; 5 of these genera cause human diseases and the others cause animal diseases.

- Genus *enterovirus*
- Genus *heparna* virus (Hepatitis RNA virus)
- Genus rhinovirus

All of these may cause respiratory infections, GIT infections, paralysis, encephalitis, meningitis, fever with rash and many other clinical syndromes.

- **Rhinoviruses** cause *common cold syndrome*. They cause rhinitis with fever. It is a self-limiting disease. We have more than 120 different serotypes of this virus. In one season we could be infected with more than one serotype of this virus. We are not concerned about it because it is a mild, self-limiting disease.
- **Hepatovirus (or Heparnavirus)**: Hepatitis RNA virus (i.e. Hepatitis A virus) will be discussed with the hepatitis viruses.

Genus Enteroviruses; this genus has the following viruses:

- Polio (types 1-3)
- Coxsackie A: 24 types
- Coxsackie B: 6 types
- Echoviruses (enteric cytopathic human orphan): 34 types
- Enteroviruses (types 68-71)
- Hepatitis A virus (Enterovirus 72)

Poliovirus as a prototype of enteroviruses

- It has a single-stranded, positive-sense RNA.
- It has four different viral proteins (VP_1, VP_2, VP_3, and VP4). We have 60 copies of each viral protein (i.e. we have 240 viral proteins in a single enterovirus). These proteins are structural proteins which make up the capsid.
- They are small viruses (20-30nm in diameter).
- They are non-enveloped viruses.

Diseases Associated with Enterovirus Infections

- Non-specific Febrile Illness (fever of unknown origin)
- Perinatal Infection
- Febrile Disease with Rash (we have to do differential diagnosis with Measles, Rubella, chickenpox)
- Meningitis
- Myocarditis
- Hepatitis
- Pleurodynia
- Poliomyelitis (by either Poliovirus or other viruses)

Background

The enteroviruses have been among the most intensively studied of all human pathogens. The war on poliomyelitis produced many breakthroughs (vaccine) in the science of virology.

Research on the enteroviruses has led to:

- Important discoveries in the replication of RNA viruses
- X-ray crystallographic characterization
- Fine structure mapping

There is no lipid in their structure so they are stable against treatment with ether, ethanol, and various detergents. They are also stable against gastric juices and bile salts; so they can pass through the stomach to the intestines and establish an infection there. Since they are heat and acid stable, they will stay viable for hours on surfaces.

Biological properties

- Found in feces & spread by fecal-oral route (respiratory route is another way of transmission)
- Grow in tissue culture with or without CPE
- Cause silent infections but also cause a number of important illnesses (e.g. poliomyelitis)
- Several genera of Enteroviruses can cause similar symptoms, e.g. aseptic meningitis or exanthems, but some diseases have a more specific association with a single genus, e.g., pleurodynia and herpangina.
- Isolation of Enteroviruses from the stool provides a basis for suspecting that the virus is responsible for the illness in question.

Viral Pathogenesis

- The virus enters the body through the mucosa of the oropharynx and upper respiratory tract, and then begins to multiply in the tissues around the oropharynx (*first round of replication*).
- Because the Enteroviruses are stable in acid they are able to pass through the stomach into the intestines, where they undergo further rounds of replication.
- Roughly at the same time as it reaches the intestine, the virus begins to spill into the systemic circulation. This early (primary) viremic phase is usually asymptomatic and involves fairly low titers of virus in the blood.
- During the primary viremia, tissues are seeded according to the *tropism* of the virus.
    - VP_1 determines the target tissue to be infected (e.g. Poliovirus infects neurons and Hepatitis A virus infects the liver)

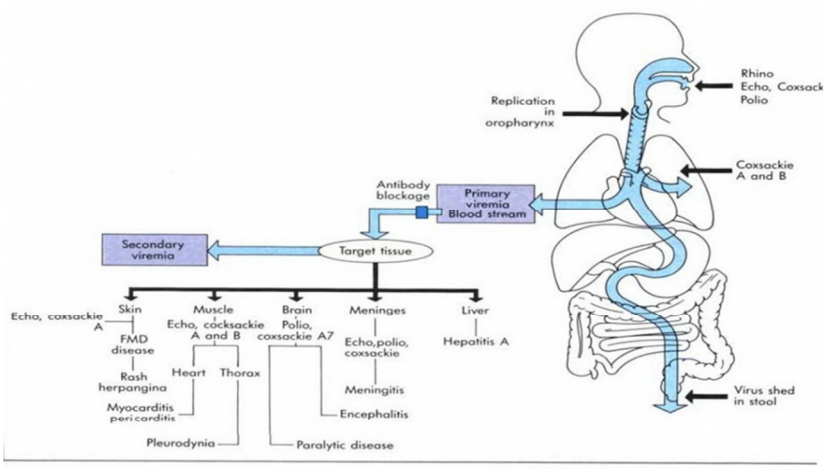


FIGURE 59-4 Pathogenesis of enteroviruses. The target tissue infected by the enterovirus determines the predominant disease caused by the virus.

Immunity

- Antibodies can be detected in the circulation by the seventh to tenth day after exposure, roughly the same time as the symptomatic disease and secondary viremia occur.
- With the exception of the gastrointestinal tract, viral replication in tissues soon slows to a halt. In contrast, gastrointestinal tract viral multiplication and fecal shedding can continue for weeks after the development of high neutralizing antibody titers.

Diseases

1. Aseptic meningitis

- **Symptoms**: headache, neck ache, rigidity of neck and back, malaise
- **Cause**: while several viruses can cause aseptic meningitis (enteroviruses, mumps, lymphocytic choriomeningitis, herpes, etc.), there are other causes of non-purulent (aseptic) meningitis (chlamydia, leptospira). Certain other bacteria and fungi may also cause non-purulent spinal fluids but with altered chemistry compared to viral meningitis.

2. Poliomyelitis

- Poliovirus was once thought to be the main cause of paralysis before the advent of polio vaccines.
- Poliovirus did account for a large portion of paralytic cases but many cases were caused by other agents or were due to unknown causes.
- The vast majority of persons infected with poliovirus have an unapparent or silent infection.
- The symptoms, locations, extent and persistence of paralysis depend on the degree of damage to the anterior horn neurons and the number of neurons affected.
- If all neurons supplying a given muscle are irreversibly damaged, the result is permanent paralysis; but if the damage to the neurons is incomplete and reversible or if some neurons are spared, the muscle function can be restored or regained.
- Paralytic disease (spinal form) may begin with excruciating pain or spasms which may precede paralysis of the extremities.
- An especially serious form is bulbar polio as it involves cranial nerves and respiratory and circulatory centers in the medulla.
- Post paralytic polio syndrome may occur many years after initial disease and reflects the continued loss of neurons with aging.

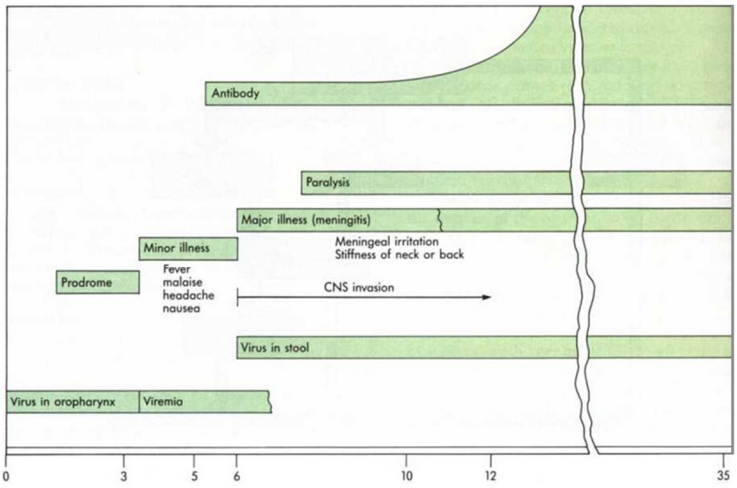


FIGURE 59-7 Progression of poliovirus infection. Polio infection may be asymptomatic or progress to either minor or major disease.

Prevention against poliovirus

- The *Salk polio* vaccine is a formalinized whole virus preparation. It is a killed vaccine given by injection. It is inconvenient that is why it is not used today.
- The *Sabin polio* vaccine is a live, attenuated virus. Attenuation means repeated passage of the virulent poliovirus in tissue culture to produce mutants which no longer are neurotrophic. The attenuated vaccine is not virulent but immunogenic. It stimulates production of IgA and IgG and it is a very effective vaccine up till now. Sabin vaccine is called a trivalent vaccine because it contains all three types of Poliovirus.
- If there is a break in the cold-chain of the vaccine or if it is subjected to heat, the virus will be inactivated. Sometimes there is reversion of the virus from the attenuated state causing the disease. These factors, along with others have caused questions to be raised about the Sabin vaccine in view of the alleged polio paralysis in a few recipients of the vaccine and their contacts.
- Immunity from **Sabin** vaccine seems to be life-long. Protection with the **Salk** vaccine requires multiple immunizations and boosters which can cause logistical problems.

Diseases Associated with Coxsackie Viruses

1. **Summer Minor Illness**: this is an acute febrile illness of short duration and without distinctive features, usually occurring in summer and fall, and may be accompanied by a rubelliform rash on the face, neck and chest.
2. **Herpangina**: mostly in children; caused by Coxsackie A (types 1-10), B (types 1-5) and some echoviruses; virus is isolated from stool in 86% of cases; epidemic in the summer months; symptoms are mild and patients recover; characterized by abrupt onset of fever, sore throat, anorexia, abdominal pain and tiny, discrete vesicles with red aureola on the anterior pillars of the fauces, the tonsils, pharynx and edges of the soft palate. (we have to do a differential diagnosis between this and measles because we have spots which resemble Koplik's spots)
3. **Pleurodynia**: Coxsackie group B; characterized by acute sudden chest pain, fever, malaise (may present as coronary occlusion); may also be accompanied by abdominal and testicular pain; viremia is followed by seeding of the virus to striated intercostal muscles; recovery is complete but relapses are common. (maybe confused with coronary heart disease)
4. **Aseptic Meningitis**: No bacteria cultivated from CNS; caused by Coxsackie A or B; fever, malaise headache, anorexia, abdominal pain and sometimes mild muscle weakness and severe stiff neck.
5. **Neonatal Disease**: Mostly group B and some group A; ranges from unapparent infection to fatal disease.(differential diagnosis with Herpes simplex virus)
6. **Respiratory Infections**: common cold-like symptoms. Caused by Coxsackie A10, A24, B3.
7. **Hand, Foot & Mouth Diseases**: vesicular lesions. Caused by Coxsackie A16, A4, A5, A9, A10.
8. **Myocardiopathy**: Involves several Coxsackie B types.
9. **Sudden Onset Diabetes**: associated with Coxsackie B4 infection.

Adenoviridae family

Introduction

They are medium-sized (70 – 90nm), non-enveloped, icosahedral (252 capsomeres, 240 hexameres, 12 pentameres) viruses with a linear double-stranded DNA. Each pentamere of the virus has a protein fiber with a knob on it. This fiber determines the tropism of that adenovirus and determines what kinds of cells will be affected because this fiber has attachment proteins for different organs in our body systems.

There are many serotypes and groups of adenoviruses with a broad spectrum of clinical manifestations. In the adenovirus family we are concerned with the genus *Mastadenovirus* which cause human infections. This virus has been accidentally isolated from adenoid tissue in 1953. The family of adenoviridae has been divided into six **subgroups** (A-F) according to the *hemagglutination* characteristic feature of the virus. In every subgroup we have different **serotypes** according to the *antigenic determinant* of the virus. The subgroup F (serotypes 40, 41) is called *enteric adenoviruses* that cause gastroenteritis.

Pathogenesis

- According to the cell tropism the virus can infect mucoepithelial cells of the:
  - Respiratory tract (like herpes simplex and varicella zoster)
  - GI tract
  - GU tract
- It enters via epithelium, replicates and spreads to lymphoid tissue
- It can go latent like herpes virus
- Viremia occurs
- We will have secondary involvement of viscera

Replication cycle

The viral DNA enters the nucleus and replicates and we have two stages in the replication cycle of adenoviruses

- **Primary (early) replication cycle:** early transcription and translation of mRNA occurs to form structural and non-structural proteins.
- **Late replication cycle:** late transcription and translation of mRNA occurs to form structural proteins (capsomeres, hexameres, pentameres, and the fiber protein)
- During the replication we have the formation of *inclusion bodies* (like herpes virus) which represent the excess amount of protein that has been formed during the replication cycle. This excess protein does not encapsidate a genome to form a virus particle but it accumulates in the nucleus to form *inclusion bodies*. These inclusion bodies can be stained with special stains and visualized with ordinary microscopes.

Types of infection

- **Lytic infection:** leads to lysis of the cell because it is a non-enveloped virus.
- **Latent infection:** the virus remains in the host cells in the lymphoid tissue (especially subgroup B, C) and it will be reactivated when there is immune-suppression.
- **Oncogenic transformation:** some strains cause cancers in lab animals (e.g. hamsters) and not human-beings

NOTE: Adenovirus can be used as a vector in gene therapy. The desired gene which is deficient in the patient is transferred into the core of the adenovirus. After that they infect the human-being with the virus, so the deficient gene will be easily expressed (*replaced*) after replication of the virus.

Physiochemical properties

- They are stable in the environment
- Relatively resistant to various disinfectants (e.g. Alcohol, detergents, chlorhexidine) because they don't contain lipid envelope
- Stable in GI tract- can withstand low pH, bile acids and proteolytic enzymes
- Incubation period is 2-14 days, varies according to the strain of the virus
- Infectivity of the virus will continue for weeks, even after recovery
- We may have secondary attack due to presence of so many different serotypes. So if we are infected with one serotype, we will be infected with other serotypes.
- We may have endemic, epidemic, sporadic or subclinical infections, highest percentage being *subclinical*
- Epidemics occur in crowded conditions e.g. schools, institutions, military, swimming pools, hospitals

Transmission

- Person-to-person, aerosols, fomites
- Respiratory secretions, tears, fecal/oral
- Under-chlorinated swimming pools, shared towels
- Under-sterilized medical equipment (eye exam equipment, etc.)

Clinical syndromes

Broad spectrum syndrome; it can be respiratory, eye, GIT, GUT infections

- **Respiratory** (serotypes 4, 7): fever, tracheobronchitis, pneumonia
- **Eye**:
  - **Pharyngoconjunctival fever**: headache, fever, malaise $\rightarrow$ epidemic in summer from contaminated swimming pools
  - **Keratoconjuctivitis**: pink or red eyes, irritation, tearing, foreign body sensation, occular pain, photophobia, fever, malaise $\rightarrow$ maybe epidemic, from contaminated swimming pools and ophthalmic solutions
  - **Acute follicular conjunctivitis**
- **Gastroenteritis**: especially in children, by subgroup F (40, 41), maybe co-infection with Rota virus, incubation period is 3-10 days, diarrhea for 10-14 days, fever, intussusception, mesenteric adenitis, appendicitis
- **GU tract:**
  - Acute hemorrhagic cystitis associated with fever, dysuria, and hematuria. More common in males than females.
  - Orchitis, nephritis, cervicitis, urethritis
- **Others:** Myocarditis, pericarditis, meningitis, rash, arthritis

Infections in immunocompromised patients

- Disseminated, severe and often fatal infections
- Due to new infection or reactivation of latent virus
- Prolonged infections with prolonged viremia and viral shedding
- Necrotizing pneumonia, hepatitis, rash, DIC, CNS involvement

Diagnosis: depends on the clinical picture

- **Respiratory infection**: nasopharyngeal swab
- **Eye infection**: conjuctival swab
- **GIT infection**: stool specimen
  - The specimen should be transported by a special transport medium in a test tube to keep the virus viable until it reaches the lab for processing
  - The specimen can be cultured in HeLa cells *tissue-culture*, human embryonic kidney cells *tissue-culture*, and shell vial *tissue-culture*
  - We can stain these cultures with **DFA** (Direct Fluorescent Antibody technique)
- **PCR**
- **Immune Electron Microscopy**: especially for stool specimens
- **ELISA**: for *antibody* detection in serum, or *antigen* detection in the specimen

Prevention

- **Hygiene** (good hand washing, contact precaution, water chlorination, sterilization of ophthalmic equipment)
- **Vaccine**: previously there was a vaccine but now it is halted due to side effects such as intussusception and reactivation because it was a live attenuated vaccine