VIROLOGY - CHAPTER TEN

PICORNAVIRUSES - PART ONE

ENTEROVIRUSES AND GENERAL FEATURES OF PICORNAVIRUSES

Dr Richard Hunt

Picornaviruses are the small positive strand RNA viruses that do not have a lipid membrane. They have a naked nucleocapsid that is about 30mm in diameter. Pico means *small*, hence small RNA viruses or picornaviruses.

Based on a number of properties including sequence homologies and acid sensitivity, there are nine genera within the *Picornaviridae*. Five of these infect humans:

- Enteroviruses
- Rhinoviruses
- Hepatoviruses
- Parechoviruses
- Kobuviruses

Parechoviruses were formerly classified among the Echoviruses and cause gastrointestinal and respiratory tract infections, and occasionally cases of encephalitis and flaccid paralysis. Kobuviruses also cause gastoenteritis.

CASE REPORTS

Poliovirus Infections in Four Unvaccinated Children --- Minnesota, August--October 2005

Human Parechovirus 3 and Neonatal Infections - 2001

Table 1 Genera of Picornaviruses

Genera that infect humans	
Enterovirus Polio Coxsackie A and B Echo Other enteroviruses	Diseases of the human (and other) alimentary tract (e.g. polio virus)
Rhinovirus	Disease of the nasopharyngeal region (e.g. common cold virus)
Hepatovirus	Human hepatitis virus A
Parechovirus	Formerly echoviruses 22 and 23. Disease of alimentary and respiratory tract
Kobuvirus	Aichi virus is the type species
Genera that infect other animals	
Cardiovirus	Mainly found in rodents Murine encephalomyocarditis, Theiler's murine encephalomyelitis virus
Aphthovirus	Foot and mouth disease in cloven footed animals

Erbovirus	The *Erbovirus* genus has a single species, *Equine rhinitis B virus*. It is divided into two serotypes
Teschovirus	From Teschen disease in pigs - virulent porcine polioencephalomyelitis which has high morbidity and mortality
Others	Drosophila C virus, cricket paralysis virus

Table 2 Enteroviruses

Virus family	Serotypes
Polio	1 - 3
Coxsackie A	1 - 22, 24
Coxsackie B	1 - 6
Echovirus	1 - 9, 11 - 27, 29 - 34
Hepatitis A	Enterovirus 72
Other Enteroviruses	68 - 71

Table 3 Properties of Rhino- and Entero-viruses

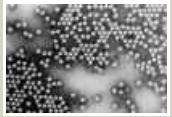
	pH sensitivity	Optimum growth temperature	Detergent sensitivity	Serotypes	Transmission	Site of primary infection
Rhino viruses	labile to acid pH	33 degrees C (approx)		>100	aerosol	upper respiratory tract
Entero viruses	resistant to acid pH	37 degrees C (approx)	Resistant	72	oro-fecal	gut

GENERAL FEATURES OF PICORNAVIRUSES

Picornaviruses have an icosahedral nucleocapsid (figure 1). There are 60 identical subunits (vertices) which contain five protomers. Each protomer is made up of one copy of four proteins, named VP1, VP2, VP3 and VP4. These proteins are made as a single polypeptide (polyprotein) which is cleaved by cellular proteases. The order of formation of the individual viral proteins is important in the assembly of the virus. The single strand of positive-sense RNA (messenger RNA sense) can act as a messenger RNA once it enters the cytoplasm and uncoating has occurred. The polio virus RNA comprises 7741 bases with a large 5' leader sequence of 743 bases that does not code for viral protein (untranslated region). The open reading frame then extends to near the 3' end. After the open reading frame of 7000 bases, there is a short sequence before the poly A tract. The poly A tract of polio RNA is encoded in the genome, unlike the situation with cellular mRNAs where it is added post-transcriptionally. There is another way in which picornavirus RNA differs from a typical mRNA. The latter have a methylated cap structure at the 5' end, whereas picornaviruses have a viral protein called VPG. The large 5' leader sequence has considerable secondary structure that comes about by intramolecular base pairing and one of these structures is the internal ribosome entry site (IRES) which allows this RNA to bind to cytoplasmic ribosomes. In the normal cellular process, initiation of protein synthesis is different and follows what are known as the Kozak rules. The initiation AUG codon in the polio virus open reading frame is preceded by eight other AUGs.

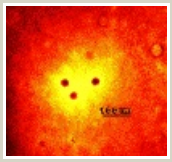

Poliovirus
© Jim Hogle, From Grant, R.A., Cranic, S. and Hogle, J.M. (1992) Radial Depth Provides the Cue. Curr. Biol. 2: 86-87. From Virus World, Sgro, J-Y

Poliovirus © J-Y Sgro, Used with permission. From Virus World


Transmission electron

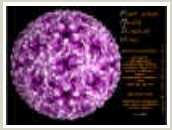
Protein synthesis initiation

micrograph of poliovirus type 1. CDC/Dr. Joseph J. Esposito jje1@cdc.gov



Negatively stained preparation of a typical Enterovirus, Coxsackie B, and seen by transmission electron microscopy. Wadsworth Center, New York State Department of Health

Cardiovirus: Molecular surface of Mengovirus, radially depth cued, as solved by X-ray crystallography © J-Y Sgro. From: VirusWorld. Used with permission



Aphthovirus: Molecular surface of Foot and Mouth Disease Virus, radially depth cued, as solved by X-ray crystallography © J-Y Sgro. From: VirusWorld. Used with permission

Figure 1 - Micrographs of picornaviruses

Receptor binding

Different picornaviruses have different receptors, among which are some intercellular cell surface adhesion molecules (ICAMs). The expression of these molecules determine tissue tropism. Coxsackievirus (a type of enterovirus, see later) and most rhinoviruses bind to ICAM-1, an adhesion glycoprotein expressed on the surfaces of a variety of cells (epithelial, endothelial, fibroblasts). Polio virus binds to another cell surface glycoprotein known as CD155 (the poliovirus receptor). When the virus binds to its receptor, the VP4 protein is released from the protomer. This allows the escape of the viral RNA from the nucleocapsid when the virus in internalized into the endocytic pathway. In the endosome, the nucleocapsid disassembles in the acid environment. Protein synthesis is detectable with 15 minutes of infection.

Translation and protein processing

The picornavirus RNA binds to ribosomes and makes a single polypeptide, therefore the virus has just one gene. This polyprotein has regions that have proteolytic activity (they are cysteine proteases) that cleave the polyprotein to three precursor proteins (P1, P2, P3). P1 is cleaved to a VP0, VP1 and Vp3 plus a leader peptide of unknown function. VP0 gives rise to VP2 and VP4. P2 and P3 do not give rise to viral structural proteins. One of the proteins that comes from P3 is the VPG that is found at the 5' end of the viral RNA while other proteins from this precursor are the viral replicase and enzymes that modify the behavior of the host cell. P2 is also cleaved to give other cell-modifying proteins. Details of some of the cleavages are still vague.

Once the various viral proteins have been made in the infected cell, the replicase (also call a transcriptase or protein 3Dpol) copies the viral plus sense RNA to negative sense RNA. Other viral proteins are also involved in this process. As new positive strand RNAs are made, they can also be translated into more viral protein. There may be as many as half a million copies of viral RNA per cell. Some of the proteolytic events outlined above take place as the nucleocapsid is assembled. This is especially the case with the VP0 cleavage to VP2 and VP4. P1 protein is the precursor that gives rise to the four structural proteins of the nucleocapsid. Five copies of P1 first associate. Endoproteolysis then occurs to form VP0, VP1 and VP3. Twelve of these pentamers than associate to form an empty capsid (procapsid). The viral RNA now associates with the capsid and at the same time, VP0 is cleaved. Release is by lysis of the host cell.

At the same time as viral protein synthesis is occurring, host cell protein synthesis is shut off. The host cell mRNAs however remain fully functional when assayed in an experimental system, so selective degradation of cell mRNAs is not the reason for protein synthesis inhibition. One way host cell protein synthesis occurs is via the cleavage of initiation factor eIF-4, one of the cap binding proteins of the host cell's ribosomes so that cellular mRNAs cannot bind to the ribosomes. Association with cap-binding proteins is a prerequisite for the translation of most cellular RNAs. Thus, only uncapped messages such as that of the picornavirus are translated. Note that most viruses express capped RNAs similar to normal mRNA and so this mechanism of shutting down host protein synthesis is not available to them. The viral proteins also change the permeability of the host cell, altering the ionic composition of the cell and inhibiting cell mRNA association with ribosomes. Moreover, the large number of copies of viral RNA simply out-compete the cell's mRNAs.

ENTEROVIRUSES

PATHOLOGY

Table 4 Human diseases caused by enteroviruses

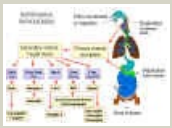
	Poliovirus	Coxsackie A virus	Coxsackie B virus	Echovirus	Enterovirus (other)
Asymptomatic infection	yes	yes	yes	yes	yes
Meningitis	yes	yes	yes	yes	yes
Paralysis	yes	yes	yes	yes	?*

Febrile exanthems	no	yes	yes	yes	yes
Acute respiratory disease	no	yes	yes	yes	yes
Myocarditis	no	yes	yes	yes	no
Orchitis	no	no	yes	yes	no

* Enterovirus-D68 (EV-D68) can replicate in blood and may damage the central nervous system. It has been detected in cerebrospinal fluid of patients with acute flaccid paralysis.

There have been reports of children hospitalized with muscle weakness or paralysis, usually in their arms and legs. They were tested for poliovirus, West Nile virus, and enteroviruses. About half of the children had EV-D68 in their nose secretions; usually, EV-D68 affects the respiratory system and it is not yet known if this respiratory infection is linked to their muscle weakness.

Enteroviruses are spread via the fecal-oral route. The ingested viruses infect cells of the oro-pharyngeal mucosa and lymphoid tissue (tonsils) where they are replicated and shed into the alimentary tract.. From here they may pass further down the gastrointestinal tract. Because of the acid stability of these viruses, they can pass into the intestine and set up further infections in the intestinal mucosa. The virus also infects the lymphoid tissue (Peyer's patches) underlying the intestinal mucosa. At these sites, the virus replicates and are shed into the feces, often for months after the primary infection. In the primary viremic phase, the virus also enters the bloodstream at low levels. The tissues that are then infected depend on the expression of the correct receptors. For example, CD155, the polio virus receptor, is expressed in spinal cord anterior horn cells, dorsal root ganglia, skeletal muscle, motor neurons and some cells of the lymphoid system. Expression of CD155 within embryonic structures giving rise to spinal cord anterior horn motor neurons may explain the restrictive host cell tropism of polio virus for this cellular compartment of the central nervous system. There are three polio virus serotypes and all of them bind to the CD155 receptor protein. For unknown reasons, polio virus does not spread to the cells of the central nervous system in all patients. The Coxsackie virus receptor (which also binds adenovirus) is a surface protein with two immunoglobulin-like domains is more widely expressed.



Pathogenesis of enteroviruses. Cox = Coxsackie virus A or B, Hep A = hepatitis A virus, Echo = echovirus, Polio = poliovirus

Figure 2 - Enterovirus pathogenesis

At this stage symptoms may occur and the patient may experience fever and malaise. A secondary viremia may occur at this time. The spread of the virus form the gastro-intestinal tract and the secondary viremia that occurs about 10 days after the initial infection leads to a humoral and cell-mediated immune response (the latter being of less importance). This rapidly limits the further replication of the virus in all tissues except the GI tract because the virus must pass through extracellular space to infect another cell. In the GI tract replication may be sustained for several weeks even though a high titer of neutralizing antibody is achieved. The cells in which this replication occurs are not known and it is unclear why replication occurs in the presence of the neutralizing antibody. Although each group of enteroviruses share a receptor, the various serotypes of a group are usually not blocked by group-specific antibodies even though it would be expected that they would have a common receptor binding site. The v reason for this appears to be that the cell receptor protein binds to a viral protein at the bottom of a canyon into which the cell protein can fit but an antibody cannot.

DISEASES CAUSED BY ENTEROVIRUSES

Most patients infected with an enterovirus remain asymptomatic but in small children benign fevers caused by unidentified enteroviruses are relatively common (non-specific febrile illness). Many outbreaks of febrile illness accompanied by rashes are also caused by enteroviruses.

POLIOVIRUS

Poliomyelitis means inflammation of the gray (*poliós*) spinal cord (*myelós)*. It is also known

as infantile paralysis. Our first record of poliomyelitis comes from an Egyptian stele from the 18th dynasty (1580-1350 BCE) showing a victim of the disease with a withered leg (figure 3).

Poliovirus caused about 21, 000 cases of paralytic poliomyelitis in the United States each year in the 1940's - 50's prior to the introduction of the Salk (inactivated) and Sabin (attenuated) vaccines. The height of the epidemic occurred in 1950 when there were 34,000 cases. By 2000, the number of cases of paralytic polio in the US was fewer than 10 and these were the result of the attenuated (Sabin) vaccine reverting to virulence (see Vaccines). Today, the attenuated vaccine is no longer used and the number of vaccine-associated polio cases in the US is close to zero. However, the ease with which the attenuated virus reverts to virulence, as a result of genetic drift (mutation), means that if people who were vaccinated with the attenuated live virus continue to shed it in feces, the problem of vaccine-associated disease will remain. Most people clear the attenuated strain of virus but a few people with immunological problems do not; for example, people with hypogammaglobulinaemia (a B-cell deficiency disorder) do not mount a humoral antibody response to poliovirus. They become asymptomatic chronic long-term excreters of the vaccine-derived virus (in one case for more than 20 years) and their virus can infect people who have not been vaccinated or who have lost immunity.

WEB RESOURCES

CDC - Polio
pdf

There are three serotypes of polio virus. Most disease results from type 1 polio virus. Since the disease is spread by fecal contamination, infections are more common where unsanitary conditions prevail but many children in these areas have asymptomatic infections that lead to life-long immunity. In contrast, in western countries naturally acquired immunity as a result of asymptomatic exposure is reduced and subsequent exposure to the virus may lead to severe disease in later life. Ironically, therefore, the disease of polio (as opposed to infection) is a disease of development and better sanitation.


Egyptian stele from the 18th dynasty showing a victim of polio with a withered leg

Figure 3

Asymptomatic polio infection

Infection by polio virus is, in most cases (more than 90%), asymptomatic. This occurs when the replication of the virus is restricted to the gastro-intestinal tract (as is the case with the attenuated vaccine strain). Exactly why many polio infections are asymptomatic is controversial but probable variables include the size of the innoculum of the virus, the size of the resulting viremia, the virulence of the infecting virus, and the presence of circulating antibodies. It is clear from clusters of cases that the same virus can cause very different outcomes in different patients from no symptoms to mild fever with diarrhea to flaccid paralysis.

Abortive poliomyelitis (minor illness)

The first symptomatic result of polio infection is febrile disease and occurs in the first week of infection. The patient may exhibit a general malaise which may be accompanied by vomiting, a headache and sore throat. This is abortive poliomyelitis and occurs in about 5% of infected individuals

Non-paralytic poliomyelitis

Three or four days later a stiff neck and vomiting, as a result of muscle spasms, may occur in about 2% of patients. This is similar to aseptic meningitis. The virus has now progressed to the brain and infected the meninges.

Paralytic polio

About 4 days after the end of the first minor symptoms, the virus has spread from the blood to the anterior horn cells of the spinal cord and to the motor cortex of the brain. The degree of paralysis depends on the which neurons are affected and the amount of damage that they sustain. The disease is more pronounced in very young and very old patients. In spinal paralysis one or more limbs may be affected or complete flaccid paralysis may occur (figure 4). In bulbar paralysis cranial nerves and the respiratory center in the medulla are affected leading to paralysis of neck and respiratory muscles. There is no sensory loss associated with the paralysis. The degree of paralysis may increase over a period of a few days and may remain for life or there may be complete recovery over period of 6 months to a few years. In bulbar poliomyelitis, death may also ensue in about three quarters of patients, especially when the respiratory center is involved. Patients were able to survive for a while using an iron lung to aid respiration (figure 4). The morality rate of paralytic polio is 2-3%


Iron lung ward in the 1950's


Paralyzed child in an iron lung


Child with polio sequelae © WHO


Victims of paralytic polio © WHO

Figure 4 - Paralytic polio

Post-polio syndrome

VIDEO

History of Polio and Polio Vaccines

Real Video
Immunization Action Coalition

This afflicts victims of an earlier polio virus infection but the virus is no longer present. It may occur many years after the infection and involves loss of function in affected muscles, perhaps as a result of further neuron loss.

COXSACKIE VIRUSES

There are many infections caused by Coxsackie viruses, most of which are never diagnosed precisely. Coxsackie type A usually is associated with surface rashes (exanthems) while type B typically causes internal symptoms (pleurodynia, myocarditis) but both can also cause paralytic disease or mild respiratory tract infection. The latter can be caused by several Coxsackie virus types and by Echoviruses and the symptoms are much like a rhinovirus infection.

Meningitis

Enteroviruses are the major cause of viral meningitis. Both Coxsackie virus A and B can cause aseptic meningitis which is so-called because it is not of bacterial origin. Viral meningitis typically involves a headache, stiff neck, fever and general malaise. Lymphocyte pleocytosis of the cerebrospinal fluid is often observed. Most patients recover from the disease unless encephalitis occurs although there may be mild neurological problems. The disease is most prevalent in the summer and fall.

Herpangina

Coxsackie virus A can cause a fever with painful ulcers on the palate and tongue leading to problems swallowing and vomiting. Treatment of the symptoms is all that is required as the disease subsides in a few days. Despite its name, the disease has nothing to do with herpes or the chest pain known as angina.

Hand, foot and mouth disease

This is an exanthem (that is, a rash) caused by Coxsackie type A16. Symptoms include fever and blisters on the hands, palate and feet. Again, it subsides in a few days. Many other exanthems may be caused by Coxsackie virus or Echoviruses.

Myocarditis

Coxsackie virus A and B (and also Echoviruses) can cause myocarditis in neonates and young children. Fever, chest pains, arrhythmia and even cardiac failure can result. Mortality rates are high. In young adults, an acute benign pericarditis may also be cause by Coxsackie viruses.

Bornholm disease (Pleurodynia, the Devil's Grippe)

Usually caused by Coxsackie A, these upper respiratory tract infections can result in fever and sudden sharp pains in the intercostal muscles on one side of the chest. There may also be pain in the abdomen and vomiting. The incubation period is 2 to 4 days and symptoms subside after a few days although relapses can occur.

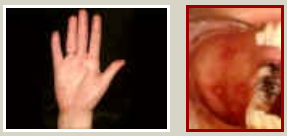
Other enterovirus diseases

Non-specific febrile disease can be caused by several enteroviruses These infections are among the most common reasons that small children are admitted to hospital in order to rule out a bacterial cause. The virus can spread transplacentally or from maternal fecal material and is most severe in infants born to mothers who contract the viral infection shortly before giving birth or in infants who contract the virus after birth. This is because the mother has not had time to developing a protective immune response and pass protective antibody to the infant. Admissions peak in the late summer/fall. Disease normally resolves but can be of consequence in the very young. Coxsackie B virus may result in severe neonatal disease including hepatitis, meningitis, myocarditis and adreno-cortical problems. Infections often spread through nurseries and are difficult to stop because of the resistance of the virus to disinfecting agents.

Acute hemorrhagic conjunctivitis is caused by Coxsackie A24 and enterovirus 70. The disease resolves in a week or two.

WEB RESOURCES

CDC - Hand, foot and mouth disease



Hand, Foot and Mouth Disease

Figure 5

Hepatitis

Hepatitis A virus, the major cause of viral hepatitis, is also an enterovirus but it will be dealt with in the hepatitis section.

PREVENTION OF PICORNAVIRUS DISEASE

One of the more important feats of 20th century medicine was the development of highly effective vaccines that have almost eradicated poliomyelitis from the world. Vaccination is therefore the major means of control of this virus and the major vaccines are discussed in the section Vaccines.

There are no vaccines for Coxsackie virus or other enteroviruses. In most cases, enterovirus infections are not life-threatening and management of symptoms are all that is required. However, certain patients particularly those with deficient humoral immunity, acquire serious infections. These include chronic enterovirus meningoencephalitis, neonatal enterovirus sepsis, myocarditis, vaccine- associated or wild-type polio virus infection, post-polio muscular atrophy syndrome, enterovirus encephalitis and bone marrow transplanted patients with an enterovirus infection. Treatment with antibody preparations (immune globulin) has resulted in stabilization of the conditions of some of these patients but the virus persists and few of these patients survived their infections. Recently, however, treatment with pleconaril (related to the WIN drugs) has shown a response that is temporally related to therapy (for further information on pleconaril and the WIN drugs, see anti-viral chemotherapy).

DIAGNOSIS

It is frequently difficult to diagnose an enterovirus disease from symptoms alone and epidemiology is used. For example, if there is a local outbreak of viral meningitis in the summer or fall, the patient is likely to be infected with Coxsackie A or B.

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