

## Families Togaviridae and Flaviviridae

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Jan.29, 2008

### 1. Representative viruses of these two families

Togaviridae:

genus alphavirus: EEE, WEE, VEE, Sindbis, Semliki forest viruses.

genus rubivirus: rubella virus

Flaviviridae:

genus flavivirus: dengue, yellow fever, St. Louis encephalitis, Japanese encephalitis, West Nile viruses.

genus pestivirus: bovine viral diarrhoea virus (BVDV).

genus hepacivirus: hepatitis C virus

### 2. Transmission in nature

-almost all of the alphaviruses are transmitted by mosquitoes, but there are a few exceptions.

-viruses in the genus flavivirus are transmitted by mosquitoes or ticks, depending on the virus.

### 3. Structural features of the viruses

1) size: alphaviruses about 70 nm vs about 60 nm for flaviviruses.

2) spherical enveloped spiked structures

3) icosahedral structure

### 4. Viruses contain 3 proteins: C and 2 envelope proteins

-the two envelope proteins are glycoproteins and are anchored in the lipid bilayer

### 5. Viral RNA:

1) ss, (+) polarity, infectious. alphavirus RNA 11,700 nt vs 10,300 nt for viruses in the genus flavivirus.

2) 5' termini of alphavirus RNA is capped, as is RNA of viruses in genus flavivirus.

-but RNAs of pestiviruses and of HCV do not have a 5' cap, they have an IRES.

3) Togavirus RNA is polyadenylated; Flavivirus RNA is not

### 6. Virus Entry:

by receptor mediated endocytosis

Two papers in Nature, Jan. 22, 2004 deal with the entry of dengue virus, and Semliki forest viruses, and conformational changes in the E and E1 protein respectively.

## 7. Release of virus from cell:

- 1) alphaviruses bud at plasma membrane, at sites where the plasma membrane (PM) has been modified by insertion of envelope proteins.
- 2) flaviviruses mature at intracytoplasmic membranes,

## 8. Overview of strategy of replication:

- 1) replication is entirely cytoplasmic
- 2) genome replication resembles that of poliovirus  
(+) strand → (-) strand → (+) strand
- 3) but the togaviruses have a variation on the basic strategy
- 4) note the different location of the genes for the structural proteins
  - with the Flaviviridae, these genes are 5' as with poliovirus.
  - With Togaviridae, however, these genes are 3'.
- 5) Togaviruses make a subgenomic RNA which is the message for the structural proteins
  - this is made off the (-) strand template
  - makes it possible to up regulate synthesis of structural proteins, relative to the non-structural proteins.
  - thus in infected cells, Togaviruses make 2 mRNA's
  - sequence of the subgenomic RNA is identical to that of the 3' 1/3 of the genome.

## 9. Synthesis and processing of structural proteins from Togavirus subgenomic (SG)RNA

- 1) The structural proteins, C, E2, and E1 are synthesized as a polyprotein.
- 2) C, or capsid protein is N-terminal and has protease activity which cleaves itself off from remainder of polyprotein.
  - the capsid proteinase is a serine protease: H-141, D-163, and S-215 represent the important catalytic amino acids.
  - The N-term. domain is not conserved among the alphaviruses and is rich in Lys and Arg.
  - C-term. domain is highly conserved and constitutes the protease.
  - bond to be cleaved (at the C-terminus of the C protein) lies right in the active site of the enzyme.
- 3) the other cleavages of this polyprotein are carried out by cellular proteases, including that of pE2→E2, which is a late cleavage.
- 4) E1 and pE2 heterodimerize and move through RER, Golgi, and to the plasma membrane.
- 5) At some point, heterodimers trimerize, into the viral spikes. The virus has 80 spikes.
  - thus each particle has 240 copies of each E protein.
- 6) Functions of E proteins: E2: attachment, E1: fusion.

## 10. Synthesis and function of the Togavirus non-structural (ns) proteins

- 1) these proteins are translated from the genome size RNA: Note that there are several stop codons in the "junction region", where the open reading frame (ORF) encoding the ns proteins, and the ORF encoding the structural proteins meet.
- 2) The ns proteins are also synthesized as a polyprotein.
- 3) how is the polyprotein processed?
  - by a viral protease, in the C-terminal domain of nsP2
  - this protein also has an RNA helicase function
- 4) nsP4 is the RDRP
- 5) nsP3 is the only one of these proteins that is phosphorylated, but its function is not clearly defined.
- 6) nsP1 is a methyltransferase. It is actually involved in both the capping (guanylation) and methylation of the 5' end of the viral mRNA's.

\*\*\*Note: Cytoplasmic viruses that make capped mRNA's have to encode the proteins that carry out the required activities.

## 11. Processing and Functions of the flavivirus proteins.

- 1) The situation here is like that with poliovirus: There is one long ORF, and it encodes one large polyprotein.
- 2) The coding information for the structural proteins is at the 5' end of the genome.
- 3) There are 3 structural proteins: C, E, and prM. E corresponds to the Togavirus E1 protein, in that they both have fusion domains. But the flavivirus E protein is also the attachment protein.  
prM corresponds to E2, and is a chaperone protein.  
What is the role of these chaperone proteins?  
To prevent premature activation of the fusion protein, during the assembly and maturation of the virus, specifically as the proteins pass through low pH environments.  
The cleavage of prM to M, like that of the Togavirus pE2 to E2, is a late cleavage.
- 4) The cleavages that gives rise to the structural proteins, are carried out by cellular proteases, but most of those that give rise to the ns proteins are carried out by a viral protease.
- 5) NS3 is the viral protease, but it also has an RNA helicase domain, and demonstrated helicase activity.
- 6) NS5 is the RDRP
- 7) NS1 is a very interesting protein: It is a ns protein, but it is glycosylated. It is quite unusual for ns proteins to be glycosylated.  
Also, Ab to NS1 are prominent after infection e.g. dengue, and these Ab are protective. It is also very unusual for Ab to a ns protein to be protective.  
Finally, the function of NS1 is poorly understood, although recent work

implies that it is required for RNA synthesis.

## **12. Infectious Plasmids of small positive-strand viruses**

These are plasmids which replicate in *E.coli* but which contain not only sequences which are required for replication of the plasmid DNA and plasmid selection, but also a complete cDNA copy of the viral genome, e.g. Sindbis virus, or dengue virus.

Upstream of the viral cDNA is a phage promoter e.g. T7 or SP6.

The plasmid also contains a restriction site downstream from the viral cDNA sequence such that the plasmid can be linearized.

When phage RNA polymerase, e.g. T7 is added to the linearized plasmid DNA the viral cDNA is transcribed into viral RNA. Addition of a cap analogue results in formation of a 5' cap on the viral RNA.

Transfection of cells with this RNA results in production of virus.

Infectious plasmids make it possible to work with an RNA virus at the level of DNA, enabling one to introduce mutations into the RNA genome and to map mutations.

## **Hepatitis C Virus (HCV)**

### **Background:**

As one reads about viral hepatitis, the viruses may seem to resemble an alphabet soup. For many years, physicians distinguished only two types of hepatitis, infectious hepatitis and serum hepatitis.

Hepatitis A virus (HAV) caused what was called *infectious hepatitis*. HAV is an RNA virus in the family *Picornaviridae*. It is transmitted by the fecal-oral route. Chronic infection is unusual, and the prognosis in nearly all cases is good.

Hepatitis B virus (HBV) causes most of what used to be called *serum hepatitis*. HBV is a DNA virus in the family *Hepadnaviridae*. It is transmitted by blood, and by bodily fluids, and can also be transmitted sexually. Chronic infection may occur, and a certain proportion of patients with longstanding chronic infection go on to develop hepatocellular carcinoma.

The prognosis following HBV infection is therefore much less favorable than following HAV infection.

Although tests of donated blood for HBV dramatically reduced the incidence of HBV following blood transfusion, many cases of hepatitis following blood transfusion continued to be seen. For want of a better name, this was referred to as non A, non B or NANB hepatitis.

The etiology of NANB hepatitis was unknown. For a long time, despite much effort, no infectious agent could be isolated from patients with NANB hepatitis.

In the late 1980's, using "immune" serum from patients with NANB hepatitis and DNA recombinant methodology, investigators established that patients with NANB hepatitis contained particles with an RNA genome in their sera. Soon thereafter the

complete sequence of the RNA genome was determined. On the basis of the sequence and the organization of the viral genome, this new agent was considered to be most closely related to the viruses in the *Family Flaviviridae*. The new agent was called **hepatitis C virus or HCV**. The *Family Flaviviridae* at that time contained 2 genera, the *genus flavivirus*, (most of the viruses in this genus are arthropod-transmitted) and the *genus pestivirus*. The prototype virus in the genus pestivirus is bovine viral diarrhea virus (BVDV). The viruses in this genus cause serious disease in cattle and swine, but so far have not been reported to cause human disease.

With respect to genome organization and strategy of replication, HCV resembles viruses in the *genus, Pestivirus*, much more closely than viruses in the *genus Flavivirus*. Since until very recently there was no system for the growth of HCV in cultured cells, and pestiviruses can be readily grown in culture, the latter were often used as a model to study the replication of HCV.

**HCV** is now classified in the **genus hepacivirus** in the **family Flaviviridae**.

### **The HCV genome and strategy of replication:**

The genome is a single stranded nonsegmented RNA molecule about 9500 nt in length and has a positive-sense or messenger polarity.

The genome contains a single long open reading frame (ORF).

Thus, like the other viruses in the *family, Flaviviridae*, the viral RNA is translated into one long polyprotein, which is cleaved cotranslationally (i.e. cleavage of the polyprotein begins before synthesis of the polyprotein is finished) by cellular and viral proteases into 10 viral proteins.

There are 3 structural proteins: The C or capsid protein, along with the viral RNA forms the nucleocapsid; E1 and E2 are membrane or envelope proteins. These proteins are encoded by the 5' portion of the genome. E2 contains a binding site for CD81 which is thought to be the receptor or coreceptor on the cell surface.

The other proteins are nonstructural(ns) proteins.

NS5B is the viral RNA-dependent RNA polymerase.

NS3 contains 2 domains, a protease domain, and an RNA helicase domain.

Little is known about the functions of the other ns proteins, although it has been suggested that NS5A contains a sequence that determines the sensitivity of the virus to interferon.

The strategy of replication is similar to that of the viruses in the *genus Flavivirus* in that the incoming (+) strand RNA serves as the template for the synthesis of a genome size (-) RNA which in turn serves as the template for the synthesis of progeny (+) strand genomic RNA molecules.

The 5' terminus of the HCV RNA is not capped; instead the 5' UTR contains an IRES or internal ribosomal entry site. This is one of the ways in which HCV resembles viruses in the genus pestivirus and differs from the viruses in the genus flavivirus. As described in an earlier section, all viruses in the family Picornaviridae also have an IRES in the 5' UTR of their RNA's.

Because of the difficulty in growing HCV, extensive use has been made of "replicons". These are viral RNA molecules that encode only the ns proteins. When

replicon RNA molecules are transfected into cells, since they encode all the proteins needed for RNA replication, these replicons are able to replicate.

### **Pathogenesis:**

10 trillion viral particles produced/day/individual even in the chronic phase of infection.

HCV is thought to replicate not only in hepatocytes, but also in B lymphocytes.

HCV has been divided into 6 genotypes on the basis of the viral RNA sequences. Also there are many subtypes.

In western countries, genotypes 1a and 1b are most common, whereas in other parts of the world, e.g. Egypt, South Africa, and Asia, one sees mainly genotypes 4, 5, and 6.

Genotypes 2 and 3 respond better to therapy than does genotype 1.

Clearance of the virus is associated with strong virus-specific responses by cytotoxic T cells and helper T cells.

### **Natural Course of the Disease:**

In most cases the diagnosis is not made during the acute infection, because most individuals show little in the way of signs or symptoms at that time. However, up to 80% of infected individuals develop a persistent viremia. Thus the infection generally becomes chronic. During this time, individuals generally remain asymptomatic for a prolonged period, perhaps 20-30 years. Most chronic infections progress to hepatitis, and result in some fibrosis. 15-20% go on to develop hepatic cirrhosis. Of those individuals who develop cirrhosis, 1-4% develop hepatocellular carcinoma each year.

Coinfection with HIV increases the incidence of cirrhosis and worsens the prognosis. Coinfection with HBV also accelerates the course of the disease.

### **Diagnosis:**

**1. serologic tests for anti-HCV antibodies.** In 30-40% of infected individuals who spontaneously clear the virus, the antibodies are no longer detectable after many years. The development of these tests markedly reduced the incidence of HCV infection following blood transfusion.

Enzyme immunoassays are commonly used to test for antibodies. Immunoblot assays (Westerns) can be used for confirmation.

**2. detection of HCV RNA.** These tests can be qualitative or quantitative. They involve the use of RT-PCR, and can detect as few as 100 molecules of HCV RNA/ml. Quantitative measurement of the viral RNA load is the preferred method to assess response to treatment.

Genotyping of HCV also depends on PCR, and is useful because knowledge of the genotype can influence the choice of therapy. The most important distinction at present is between genotype 1, on the one hand, and genotypes 2 and 3 on the other hand (see above).

**3. Assessment of liver disease.** Measurement of alanine aminotransferase. Liver biopsy may sometimes be indicated.

**Treatment.**

The best available treatment is the combination of interferon- $\alpha$  and ribavirin; however less than 50% of patients will have a favorable response. The response to treatment can be measured by following the level of alanine aminotransferase, and the load of viral RNA in the serum.

Those who should be treated include individuals who have high levels of alanine aminotransferase, or HCV RNA in the serum, and evidence of liver damage and inflammation as seen in a liver biopsy.

Relapse is common after therapy is stopped.

Liver transplant is sometimes indicated; however, in most cases the liver graft will also become infected. Liver damage due to HCV infection is the most common reason for liver transplant.

**COMPARISON OF HAV, HBV, and HCV**

	<b>HEPATITIS A HAV</b>	<b>HEPATITIS B HBV</b>	<b>HEPATITIS C HCV</b>
Family	Picornaviridae	Hepadnaviridae	Flaviviridae
Genome	ss RNA (+) strand	partially ds DNA	ss RNA (+) strand
Transmission	fecal-oral	injection, body fluids	injection, body fluids, unknown
Acute disease with jaundice	common	common	uncommon
Chronic infection	very rare	common in children	> 70%
Estimated number of chronically infected persons in the US	-----	1,250,000	2,700,000

Treatment	None	interferon- $\alpha$ lamivudine	interferon- $\alpha$ , together with ribavirin
Prevention	killed virus vaccine, immune globulin post exposure	recombinant viral protein	None