Natural history of hepatitis C virus among injecting drug users
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Chapter 1

General introduction

HAV was recognized in 1965 as the causative agent of a previously unrecognized type of hepatitis. The virus was initially studied in a seroepidemiological survey of workers in a food processing plant in the United States, where a high prevalence of antibodies to HAV was found. The virus was subsequently isolated from infected individuals, and its complete genome was sequenced. HAV is a small, single-stranded RNA virus belonging to the Picornaviridae family. The viral genome encodes a single large polypeptide that is processed into four structural proteins.

HAV is transmitted primarily through the oral route, typically by ingestion of contaminated food or water. The virus is highly resistant to environmental conditions and can survive for long periods in the environment. The incubation period of HAV infection is typically 15-50 days, with a mean of 28-34 days.

Once infected, an individual becomes a source of infection for others, and the virus is excreted in the feces for several weeks after the onset of symptoms. HAV infection is highly contagious, and outbreaks of hepatitis A are common in areas with low levels of hygiene and sanitation. The virus is not transmitted person-to-person, and there is no effective treatment or vaccine for HAV at the present time.

The clinical symptoms of HAV infection are typically mild and self-limiting. The incubation period is followed by the onset of symptoms, which usually include fever, fatigue, nausea, vomiting, and abdominal pain. Jaundice may also develop, usually a few days after the onset of other symptoms. The illness is typically mild, with resolution within 2-3 weeks, although some individuals may experience a more severe course of disease.

Outbreaks of HAV infection have been reported worldwide, with outbreaks occurring more frequently in regions with poor sanitation and hygiene. The virus is most commonly transmitted through the ingestion of contaminated food or water, with shellfish and raw shellfish being common sources of infection. The virus is highly resistant to environmental conditions and can survive for long periods in the environment, making it difficult to control outbreaks.

Although there is no specific treatment for HAV infection, supportive care and hydration are important. In severe cases, liver transplantation may be necessary. Prevention of HAV infection is crucial, and efforts to improve hygiene and sanitation, as well as vaccination programs, are essential in controlling outbreaks and preventing the spread of the virus.
Chapter 1
Previous studies have shown that interferon therapy is effective in resolving HCV infection. However, recent studies have suggested that interferon therapy may not be effective in all patients, and that the response to therapy may be influenced by factors such as age, sex, and co-infection with other viral infections.

Chapter 2
Negative controls were used to confirm the specificity of the assay and to ensure that the results were not due to contamination or artifact. The assay was performed on a panel of sera from healthy donors and patients with chronic HCV infection.

Chapter 3
Further studies are needed to determine the optimal treatment regimen for HCV infection, and to identify biomarkers that can predict treatment response.

Chapter 4
The current study investigated the role of HCV genotype in determining response to interferon therapy. The study was conducted on a cohort of patients with chronic HCV infection who were treated with interferon and ribavirin.

Chapter 5
The results of this study suggest that the HCV genotype is a significant predictor of response to interferon therapy. Patients with genotype 1b were found to have a higher rate of sustained virological response compared to patients with other genotypes.

Chapter 6
Evidence for both HIV and HCV co-infection is increasing, and the role of each virus in the pathogenesis of liver disease is not fully understood. This chapter reviews the current literature on HIV and HCV co-infection.

Chapter 7
Reduction of NS3 and NS5 translation in hepatitis C virus-infected cells in HCV RNA levels in HIV-infected ingesting drug users Submitted to Journal of Infectious Diseases

Chapter 8
General discussion

Chapter 9
Acknowledgements

Appendix
General introduction

1. History
Hepatitis is an inflammatory disease of the liver that can be caused by metabolic disorders, alcohol abuse, or hepatotropic viruses actively replicating in the liver. Hepatitis and jaundice have been recognized for centuries, and the clinical features were probably first described by Hippocrates (460-375 BC) as epidemic jaundice. However, it was not until the early 1970s that serological tests for hepatitis A virus (HAV) and hepatitis B virus (HBV) became available. By now, at least six hepatitis viruses, designated A-B-C-D-E-G, have been identified.

HAV was identified in 1973 in the stools of infected patients. The infection is spread by fecal contamination of food or drinking water and has a sudden onset after a short incubation period. The agent is a small (27 nm) non-enveloped virus that contains a single-stranded RNA genome of approximately 7500 nucleotides (nt) and belongs to the Picornaviridae. The viral RNA encodes a single large polyprotein from which structural and non-structural proteins are cleaved. Like most viral infections, HAV infection is characterized by a prodromal flu-like illness followed by mild gastrointestinal symptoms like nausea, diarrhea, and jaundice. The severity of clinical illness is age-dependent, and infections among infants are usually asymptomatic. The prevalence of HAV is highest in areas of substandard hygiene and varies considerably among individuals according to socioeconomic level and sexual preference.

HBV was recognized in 1968 as the so-called Australia antigen and is efficiently transmitted by parenteral and sexual exposure. Within a decade following the discovery of Australia antigen, which was ultimately shown to be the surface antigen of HBV, the virus was fully characterized. It contains a circular, partially double-stranded DNA genome of approximately 3200 nt, replicates through an RNA intermediate, and is classified as a Hepadnavirus. HBV infection can result in a broad spectrum of outcomes, from recovery to chronic hepatitis and hepatocellular carcinoma. The virus causes clinical illness in 30-50% of all infected individuals over the age of 5 years, but a chronic infection develops in only 2-10% of cases.

Serological tests for infection with HAV and HBV were developed in 1974, but it was found that most cases of transfusion-associated hepatitis were not caused by either HAV or HBV infections and were therefore named non-A, non-B hepatitis (NANBH). In the late 1970s and early 1980s, studies of chimpanzee models led to a number of important findings related to NANBH. Numerous attempts were made to characterize and clone its viral agent, but they failed due to the lack of a tissue-culture system, any sequence information, or a serological assay. Cloning of the genome was finally achieved using a cDNA expression library derived from a pool of plasma from chimpanzees experimentally infected with NANBH. After the detection of the first NANBH specific clone (5-1-1), the entire viral genome of the agent -now termed hepatitis C virus (HCV)- was sequenced, and its structural and functional organization was defined. Although HCV was first recognized to be commonly associated with blood transfusion, it is now known to be an important cause of community-acquired viral hepatitis. The highest rates of infection (60-90%) are found among persons with repeated direct percutaneous exposures, such as hemophilia patients and injecting drug users. HCV consists of a single-stranded, positive-sense RNA genome containing approximately 9000 nt with one single open reading frame. This large open reading frame encodes one polyprotein of 3000 amino acids (aa), which is cleaved during and after translation into structural and nonstructural proteins.

Hepatitis delta virus (HDV) was discovered in 1977 and was initially described as a new antigen detectable in patients with HBV-associated chronic liver disease. Studies in chimpanzees established that HDV was transmissible but dependent on the presence of active HBV infection for its replication. The HDV is an enveloped particle of 35 to 38 nm containing a
small circular single-stranded RNA genome of approximately 1700 nt with an outer coat of the hepatitis B surface antigen (HbsAg). Surveys measuring HDV prevalence in various populations with acute or chronic HBV infection provide the most important information on its epidemiology. The clinical features of acute and chronic HDV infection are similar to those of other types of hepatitis. In general, HDV augments the severity of acute and chronic HBV infections, and HDV coinfection has been found to be five times higher than single HBV infections among patients with fulminant cases of HbsAg-positive hepatitis.

Hepatitis E virus (HEV) infections resemble HAV infections, and transmission occurs via the fecal-oral route, generally leading to an acute, self-limited and icteric disease. HEV appears to be endemic among children and young adults in India, central Asia, China, Africa, and some parts of the former Soviet Union; its transmission is associated with ingestion of fecally contaminated drinking water. HEV was cloned and sequenced in 1990 and is a virus of 32 to 34 nm containing a 7300 nt positive-stranded RNA genome with three open reading frames. It is provisionally classified under the family of Calciviridae.

Approximately 5% to 20% of cases of hepatitis are not explained by the five known hepatitis viruses and are assumed to be caused by non-A-E agents. The currently named hepatitis G virus (HGV) was isolated and identified from the plasma of a patient with chronic hepatitis, who was coinfected with HCV. HGV appeared to have a high homology of 95% at the amino acid level with HCV, suggesting that the two viruses are closely related. Blood-borne transmission of HGV appeared to be the most common route of transmission. Although the prevalence of HGV was associated with acute and chronic hepatitis and found to be higher than its prevalence in healthy blood donors, histopathologic abnormalities and severe hepatitis were not found. The features of the different known hepatitis viruses are summarized in Table 1.
Table 1. Etiologic agents of viral hepatitis and their characteristics.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Classification</th>
<th>Genome</th>
<th>Outcome of infection</th>
<th>Mode of transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAV</td>
<td>Picornavirus</td>
<td>single-stranded positive-sense RNA (7.5 kb)</td>
<td>Acute</td>
<td>Resolved</td>
</tr>
<tr>
<td>HBV</td>
<td>Hepadnavirus</td>
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<td>Chronic</td>
</tr>
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<td>HCV</td>
<td>Flavivirus</td>
<td>single-stranded positive-sense RNA (9.0 kb)</td>
<td>Acute</td>
<td>Chronic</td>
</tr>
<tr>
<td>HDV</td>
<td>Satellite virus</td>
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<td>Chronic</td>
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<tr>
<td>HEV</td>
<td>Calicivirus</td>
<td>single-stranded positive-sense RNA (7.3 kb)</td>
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<td>Resolved</td>
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<tr>
<td>HGV</td>
<td>Flavivirus</td>
<td>single-stranded positive-sense RNA (9.0 kb)</td>
<td>Acute</td>
<td>Chronic</td>
</tr>
</tbody>
</table>

2. The structural and functional organization of HCV

As noted above, HCV contains a single-stranded, positive-sense RNA genome of approximately 9000 nt, with a single large open reading frame that encodes a single polyprotein of 3000 amino acids (aa) \(^{22,23}\). This is processed into structural and nonstructural proteins by cellular proteases and a virally encoded serine protease located in the N-terminal domain of the nonstructural protein NS3.

The genome contains a short untranslated region (UTR) at each end. Structural proteins are processed from the N-terminal region of the polyprotein and form the viral particle. The N-terminal region encodes for the nucleocapsid protein (core) and two envelope glycoproteins (E1 and E2). These three structural proteins are processed from the polyprotein at least in part through the action of host's signal peptidase, which cleaves after signal sequences within the polyprotein \(^{37,38}\). The non-structural proteins (NS2, NS3, NS4, and NS5) are involved in maturation and replication of the virus. These structural and non-structural proteins show great similarities with polyproteins encoded by pestiviruses and to a somewhat lesser extent by the flaviviruses \(^{22,39}\). Moreover, the hydrophobicity profile of the HCV polyprotein is similar to that of the pestiviruses and flaviviruses \(^{22}\). Taken together, these findings indicate that HCV is a small, enveloped virus that belongs to the family of Flaviridae \(^{39}\). Figure 1 illustrates the putative functional organization of the HCV genome.
Open reading frame (± 3000 bp)

<table>
<thead>
<tr>
<th>aa no</th>
<th>1</th>
<th>192</th>
<th>384</th>
<th>810</th>
<th>1027</th>
<th>1685</th>
<th>1973</th>
<th>3011</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'UTR</td>
<td>C</td>
<td>E1</td>
<td>E2/NS1</td>
<td>NS2</td>
<td>NS3</td>
<td>NS4</td>
<td>NS5</td>
<td>3'UTR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>250 nt</td>
</tr>
</tbody>
</table>

5'UTR 341 nt 

Core protein

Envelope proteins

RNA helicase

Serine protease

Metalloprotease

Figure 1. The genomic organization of hepatitis C virus. The 5' and 3' untranslated regions flanking a single open reading frame are shown. Structural proteins (core and envelope) as well as nonstructural proteins (NS2, NS3, NS4, and NS5) and predicted functions are shown.

2.1. The 5' untranslated region
The 5' UTR extends upstream of the presumed start-codon of the polyprotein. Consisting of up to 341 nt, it is highly conserved among different HCV genotypes and is often used as the target in HCV genotyping. The strong conservation of this sequence suggests that it is involved in some aspects of viral replication. Initiation of translation may be such an involvement, because the sequence contains complex secondary structures including multiple stem-loop structures like those found in the leaders of picornaviruses, in which ribosomes are known to initiate translation internally. The HCV leader comprises three large stem-loop structures in which a pyrimidine-rich region resembles the structure in picornaviral genomes that are translated by a mechanism allowing ribosomes to bind internally on the genome and initiate translation at specific AUG codons, the so-called internal ribosome entry sites.

2.2. The core protein
The core protein forms the N-terminus of the HCV polyprotein and is the most conserved among the three structural proteins of different HCV strains. The core protein is highly basic and probably interacts with the encapsidation process of the genomic RNA. It is released from the viral polyprotein by nascent proteolytic cleavage at aa 191 by host proteases and may suppress the transcription of several host genes as well as interfere with the expression of coinfected genomes of HBV and HIV. The core protein contains a DNA-binding motif (SPRG), triplicate nuclear localization signals, and several kinase recognition sites all of which are characteristics of gene-regulatory proteins. HBV markers are suppressed by superinfection with HCV, and this inhibitory effect may occur in the process of transcription and encapsidation of pregenomic HBV RNA. Repression of HIV occurs through an HCV core protein response to a region between nucleotides -65 and +3 within the LTR of HIV. Moreover, core is able to suppress apoptotic cell death in artificial systems, and its interaction with the lymphotoxin-β receptor, which plays a role in signalling apoptotic cell death,
suggests that core may influence viral persistence and disease pathogenesis.

Finally, several highly conserved immunoreactive epitopes are present in core and different recombinant core proteins and linear synthetic peptides are used for the detection of HCV antibodies. Some of these immunodominant epitopes exhibit antigenic variation and can be used to distinguish serotype-specific antibodies and differentiate among various different HCV infections.

2.3. The Envelope proteins E1 and E2

E1 (192 aa) and E2 (430 aa) are glycoproteins and are highly divergent among different HCV isolates. E2 represents the most variable region of the HCV genome and the variation is assumed to be caused by random mutation and selection of mutants capable of escaping from neutralizing antibodies produced by the host. In particular, a region of approximately 30 aa, located at the N-terminus of E2 at aa positions 385-414, shows extensive genetic heterogeneity, called the hypervariable region (HVR1). HVR1 may be important in neutralization and immune selection because of its extreme variability caused by random mutation and selection of mutants that escape neutralizing antibodies, leading to chronic HCV infections.

A second hypervariable region (HVR2) is smaller and comprises aa positions 474-480.

The E2 protein binds with high affinity to human lymphoma and hepatocarcinoma cell lines, and furthermore, in chimpanzees vaccinated with recombinant envelope proteins, protection from homologous HCV challenge was observed and correlated with the presence of antibodies capable of inhibiting the binding of E2 to human cells. The recent discovery of the interaction of E2 with human CD81, a human cell surface protein expressed on various cell types including hepatocytes and B lymphocytes, may help to elucidate HCV pathogenesis.

The sequence heterogeneity in particular regions of the envelope may have major implications for protective immune responses and for vaccine strategies, whereas the identification of interaction between CD81 and HCV may assist the development of new therapeutic strategies that interfere with virus binding.

2.4. The NS2 and NS3 proteins

The putative nonstructural (NS) proteins of HCV appear to be processed from the polyprotein through the action of two virally encoded proteases. The first protease, which is derived from a region spanning NS2 and the N-terminus of NS3 (aa 827-1207), can be stimulated by zinc ions and appears to be a metalloprotease involved in cleavage at the NS2/NS3 junction. Remaining NS proteins are processed by the second protease, which is encoded within the NS3 region. This second protein is a trypsin-like serine protease with an active domain that overlaps the metalloprotease with distinct cleavage sites.

Besides the proteolytic enzyme activity of NS3, it comprises other motifs such as NTPase and RNA helicase activities, involved in unwinding of the double-stranded replicative intermediate necessary for replication. The NS3 region is one of the most intensively studied regions of the HCV genome because of its potential for protease-inhibitors and is used in several antibody detection assays.

2.5. The NS4 protein

The NS4 region comprises two proteins, namely NS4a and NS4b, which are released from the polyprotein by the protease activity of NS3. Although the function of NS4 is still unknown, a recently found putative tat-binding motif may be involved in the binding of the HIV tat-gene and the modulating of HCV replication. Containing several immunodominant epitopes, NS4 is used in most antibody detection assays.
2.6. The NS5 region
The NS5 region of the polyprotein is composed of two major proteins, NS5a and NS5b, which are released by the action of the NS3 protease in conjunction with NS4a. The functional role of NS5a is currently unclear but is probably involved in the replication cycle. NS5b contains an amino acid sequence motif (Gly-Asp-Asp), which is known to be highly conserved among RNA-dependent RNA polymerases, and thus it is likely that NS5b is the viral polymerase \(^{22,73}\). NS5 contains several immunodominant epitopes and is used in several third-generation antibody assays.

2.7. The 3' untranslated region
The 3' UTR is variable in length, spanning 170 to 250 nt. Its 3'-terminal sequence of 98 nt is well conserved and probably involved in genomic replication at the level of priming minus-strand RNA synthesis \(^{74,75}\).

3. Prevalence

3.1. General population
HCV infection has been found worldwide, and recent studies throughout the world have defined the prevalence of antibodies to HCV in the general population \(^{76,77}\). The estimated prevalence of antibodies to HCV in the general population of the Western world is 1-2%. Blood donors, a population highly screened for viral infections, probably shares basic characteristics with the general population but may not reflect the prevalence of antibody-status of the general population \(^{78}\). The risk of acquiring antibodies to HCV by blood transfusions was calculated as 1/100,000 transfusions \(^{79}\). Despite the best precautions this remaining low risk is believed to be caused by the window phase of the primary HCV infection, in which the immune system is not able to mount enough antibodies to be detected by antibody assays. Currently, the prevalence of HCV among blood donors is measured by detecting antibodies to HCV, but after July 1999, RT-PCR will be used in the Western world for the direct identification of HCV infection.

The prevalence of antibodies to HCV differs among populations of blood donors worldwide. Relative low rates of antibodies to HCV (0.2-1.0%) have been found in parts of Europe, North America, Australia, South Africa and parts of Asia. Higher prevalences (1.0-5.0%) have been found in Japan, the former Soviet Union, Brazil, Ethiopia and Saudi Arabia. The highest prevalences have been reported in Egypt, where up to 15% of the blood donors were found to be positive for antibodies to HCV.

3.2. Risk groups
Direct percutaneous exposures, such as transfusion of blood or blood products, transplantation of HCV-infected organs or tissues, and the sharing of contaminated needles or syringes, have been associated with the most efficient transmission of HCV. Therefore, high prevalences of HCV antibodies are found among groups such as hemophiliacs \(^{80-82}\), patients on hemodialysis \(^{83}\), transplant recipients \(^{84,85}\), and injecting drug users \(^{86,87}\). Chronic HCV infection is common in all of these groups, ranging from 50% in the first three groups to nearly 100% in injecting drug users.

4. Genetic heterogeneity: genotypes and quasispecies
HCV is an RNA virus that replicates by means of an RNA polymerase that lacks proof-reading activity and generates sequence variation comparable to influenza virus and human immunodeficiency virus. The genomic heterogeneity of HCV occurs on two different levels.
The widest variation is observed among HCV isolates of distinct genotypes due to the accumulation of mutations during long-term evolution of the virus and separation of the genotypes. In general, isolates of the same genotype or subtypes differ in sequence by 5-10%, whereas isolates of different genotypes differ by up to 35%. The narrowest variation is observed among the isolates of a single strain—the so-called quasispecies—in a single host. Within an infected individual, the HCV genomes exist as a quasispecies, usually representing one dominant species and many minor ones. These quasispecies which result from random mutation and selection by immune-pressure during the course of infection, differ from each other by 1-2%.

HCV genotypes are labeled by arabic numbers (1, 2, 3, etc.), and the related subtypes are named with lower case letters (a, b, c, etc.). Six major HCV genotypes (1-6) have been identified along with 12 subtypes, by sequence analysis of the E1 gene (576 nt) and the core gene (573 nt). The same six major genotypes were found analyzing sequences of the NS5b gene, but only 11 subtypes. Figure 2 illustrates a phylogenetic tree of the 6 major genotypes based on the analysis of a 160 base pairs sequence spanning nt -245 till -75 in the 5' UTR taken from a substantial number of published HCV isolates and our own created sequences.

The level of sequence diversity may have great implications in terms of disease progression and treatment worldwide. The worldwide distribution of HCV genotypes shows distinct geographic differences. Whereas 1a, 1b, 2a, 2b and 3a are predominant in Western Europe and in the USA and 1b, 2a, and 2b are predominant in Japan and Taiwan, HCV genotype 4 is most prevalent in the Middle East and Africa, genotype 5 in South Africa, and genotype 6 in Hong Kong. The six HCV genotypes have been associated with the finding of different histologic abnormalities in cases of chronic hepatitis, and different responses to interferon (IFN) therapy. Patients infected with genotype 1, in particular 1b, respond poorly to treatment compared with patients infected with other genotypes. In several studies, a sustained response was achieved in only 97 (18.1%) of 556 patients infected with genotype 1, compared with 158 (54.9%) of 288 patients with other genotypes. Of importance, in many of these studies, response was not defined as viral clearance but as normalization of liver functions. In chronically infected individuals, the viral load, genotype, and elevated serum alanine aminotransferases (ALT) levels may have clinical relevance.

Figure 2. Phylogenetic tree showing 6 distinct genotypes based on a 160 base pairs sequence of the 5' UTR region.
5. Clinical profile of HCV infection
In the acute phase of HCV infection, the clinical symptoms are indistinguishable from HAV and HBV infection. HCV infection progresses with an indolent course and remains benign in the first few years, but more than half of HCV-infected individuals eventually develop chronic HCV infection. Chronic HCV infection is often silent, and clinical symptoms are absent or minimal unless the disease is severe or cirrhosis is diagnosed. The clinical manifestations of HCV infection are diverse and the clinical course is difficult to predict. Histologically, the chronic disease is commonly associated with liver necrosis, scarring, fibrosis, and cirrhosis. A further important sequela of HCV infection is the gradual progression to hepatocellular carcinoma in some patients.

The natural history of HCV is poorly understood, especially the degree to which hepatocellular damage is mediated by the immune system or by the direct pathogenicity of HCV.

6. Host immune response
Like all viral infections, the host immune response to HCV comprises both humoral and cellular components. Antibodies to different HCV proteins develop during infection, and the cellular immune response is also activated in these patients, with the presence of CD4+ and CD8+ cells responding to various processed antigens.

6.1. Humoral immune response
Viral proteins trigger the B-cells to produce antibodies to both structural and non-structural proteins of HCV. The presence of antibodies to HCV is detected by immuno-assays and is closely related with infectivity, especially in blood donors, haemodialysis patients, haemophiliacs, and patients with chronic HCV. However, at least 20% of patients with HCV may not develop antibodies to HCV for weeks to months after the onset of infection and clinical illness. An additional 10% remain antibody negative, and infection in these individuals can be diagnosed only by detection of HCV RNA. The order of appearance of antibodies to HCV is erratic but the reactivity in tested population is core > NS3 > NS4 > NS5. Importantly, despite most patients develop antibodies to HCV, antibodies do not indicate protection from new HCV infection. Both humans and chimpanzees can be reinfected, even with the same strain, with or without clearance from blood of the original infection.

Most patients eventually mount a detectable antibody response to HCV infection, but immunosuppressed patients may represent an exception to this general rule. In studies of transplant patients, up to 50% fail to generate a humoral response, and individuals coinfected with HCV and HIV show impaired HCV antibody responses and increased seroreversions. This is not the case with other viral pathogens, such as herpes simplex virus (HSV), varicella-zoster virus (VZV), and cytomegalovirus (CMV), among immunosuppressed patients. This finding suggests that, compared to those viruses, HCV is less immunogenic in activating the humoral response.

6.2. Cellular immune response
The cellular immune defence is highly dependent on CD4+ T-cell responses, which can expand antibody production by B cells and prime CD8+ cytotoxic T-lymphocytes specific to virus-infected cells. Short, processed antigenic peptides presented on the cell surface are recognized by both classes of T cells. These peptides are generally presented to CD4+ T-cells by major histocompatibility complex class II molecules found on specialized antigen-presenting cells, or to CD8+ T-cells by class I molecules, expressed on virtually all cells. Two different types of T-lymphocytes exist: cytotoxic and helper. CD4+ cytokine responses are coordinated...
by CD4+ T helper (Th) cell responses Th1 and Th2. Th cells independently recognize pathogens and secrete cytokines that stimulate growth and responsiveness of B cells, T cells and macrophages. Th1 cells secrete interleukin 2 (IL-2) and interferon-γ, which are needed for generation of cytotoxic T-lymphocytes and activation of natural killer cells. Th2 cells secrete IL-4 and IL-10 required for antibody production. However, the mechanism by which CD4+ and CD8+ T-cells directly or indirectly control HCV remains to be elucidated.

7. HCV RNA levels

7.1. Profiles among patients not receiving antiviral-therapy
The illness has a complex course, with RNA levels that are often transient. HCV RNA is usually detectable within weeks after infection and followed by a peak of ALT and usually not found simultaneously at the same time-point in some acute cases. Typically, the chronic phase of infection is marked by lower RNA levels than the acute phase. Viral persistence, reflected by detectable HCV RNA and antibodies to HCV, is found in the vast majority (>80% of patients) generally with associated fluctuating ALT levels but sometimes without ALT elevations. Thus, chronic HCV infection is more frequent than indicated by ALT elevation alone. HCV RNA quantification is a direct measurement of viral replication in blood and has significant clinical implications and is superior to detection of ALT elevations but the relation to liver disease is still unclear. A burst of viral replication may be directly responsible for liver damage, either by direct pathogenicity or indirectly mediated by the immune system, by immune complex formation, or by cytotoxic T-lymphocyte responses. In some cases, HCV RNA load is directly related to increased hepatocellular damage, as characterized histologically by hepatic fibrosis and architectural distortion, but it is unclear whether viral and biochemical markers can define the risk of developing hepatocellular carcinoma.

Among high risk groups such as injecting drug users, coinfection with HCV and HIV occurs due to the parenteral route of transmission. Several reports have suggested that HCV replication is enhanced by HIV infection, either by HIV-induced immunosuppression or by HIV-HCV interaction. Whether liver disease is more severe due to HIV-coinfection is still under debate, but there is agreement that HIV-coinfected patients progress more rapidly to liver disease.

7.2. Profiles among patients with antiviral-therapy of HCV infection
In studies relating viral load with response to therapy indicate that patients with low HCV RNA levels respond to interferon in a sustained manner. Response to interferon is defined as normalization of ALT within 6 months, but the biochemical response does not always reflect the HCV RNA profile during treatment. Good sustained response is seen in 10% to 15% of patients and seems to be dependent on genotype and the emergence of quasispecies, and is transient in the majority of patients.

In a recent study, it was found that high IFN doses of 10 and 15 mIU daily had better antiviral efficacy, was inversely correlated with viral load and was positively correlated with cell death rate and ALT levels. The same study also showed that HCV infection is highly dynamic, which may have implications for the development of viral resistant quasispecies. Early monitoring of viral load and treatment with high doses of interferon may increase initial therapy efficacy.

In another study, 43% of patients were found to be sustained responders after treatment for 48 weeks with 3 mega units (MU) interferon three times a week plus 1000-1200 mg ribavirin per day. The rate was 31% to 38% when combination therapy was used as the initial treatment and 49% when it was used for the treatment of relapse. Five independent factors were signifi-
cantly associated with this good response: infection with genotype 2 or 3, viral load of less than 2 million copies/ml, patient age 40 years or less, minimal fibroses, and female sex. As has been seen in the search for therapy for HIV infection, more studies are needed to develop the most effective combination therapies for HCV.

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