MAJOR HISTOCOMPATIBILITY COMPLEX AND IMMUNE RESPONSE IN HEPATITIS-C DISEASES

ABSTRACT

The hepatitis C virus (HCV) is a member of the Flavivirus genus – positive stranded RNA viruses. Unlike many of its relatives, it is able to set up persistent infections. This coupled with lifestyle changes and a number of iatrogenic disasters have led to its recent spread worldwide. HCV is most common among endovenous drug users as well as recipients of blood products in the prescreening era. HCV persists in the majority of infected individuals and is responsible for a wide spectrum of chronic liver lesions ranging from minimal inflammation to cirrhosis or hepatocellular carcinoma. The mechanisms whereby HCV establishes persistent infection and liver disease are still poorly understood. Both virus-related factors, such as viral heterogeneity and replicative activity, and host determinants, such as lack of efficient immune response, are certainly involved in the pathogenesis of chronic hepatitis. Differences in the immunogenetic backgrounds of infected patients might in part account for the variation observed in the individual course of disease. Indeed, polymorphisms of immune regulatory genes such as cytokines or HLA class I and II molecules are known to influence the host’s ability to block or to react against viral antigens. There have been several reports describing the association between HLA and various stages of HCV infection i.e. susceptibility to infection, viral clearance and disease progression. These findings, however, were somewhat conflicting due to factors such as ethnic differences as well as population heterogeneity. As always in clinical research, study design and subject selection is crucial perhaps even more here than elsewhere.

INTRODUCTION

Although primary infection with hepatitis C virus (HCV) is generally asymptomatic and fulminating hepatic failure is rare, the viremia persists in 80% or plus of patients and it is a leading cause of chronic liver disease, cirrhosis and hepatocellular carcinoma in some patients. HCV is a member of
the Flavivirus genus – positive stranded RNA viruses, which include relatives such as the viruses causing dengue and yellow fever. Its origin is obscure and certain strains have probably been circulating in the human population for hundreds of years (Pybus et al. 2001). The World Health Organization estimates that about 170 million people, 3% of the world’s population, are infected with HCV and are at risk of developing liver cirrhosis and/or liver cancer. The prevalence of HCV infection in some countries in Africa, the Eastern Mediterranean, South-East Asia and the Western Pacific (when prevalence data are available) is high compared to some countries in North America and Europe. No vaccine is currently available to prevent hepatitis C, and treatment for chronic hepatitis C is too costly for most persons in developing countries to afford (WHO 1999). In Brazil, it is estimated that between 2.5 and 4.9% of the general population present anti-hepatitis C virus (HCV) antibodies, which corresponds to as many as 3.9 to 7.6 million chronic carriers (Brandão & Fuchs 2002).

Means of transmission.
HCV is transmitted primarily by parenteral routes. Major sources of infection include injection drug use, needle-stick accidents or reuse of contaminated instruments in health or other percutaneous procedures (e.g. ear and body piercing, circumcision, tattooing), and transfusions of unscreened blood or blood products. Maternal-fetal and sexual transmissions are relatively rare. HCV is not spread by sneezing, hugging, coughing, food or water, sharing eating utensils, or casual contact. The virus reaches the liver via the hepatic artery or the portal vein and enters and replicates in hepatocytes preferentially (Busch 2001).

The hepatitis C virus.
HCV is small and spherical (diameter approximately 30-50nm) with a lipophilic envelope. It was cloned from the plasma of an experimentally infected chimpanzee and it was characterized as a positive sense, ssRNA virus. On the basis of genome homology and hydrophobicity patterns, HCV is related to the third genus of the Flaviviridae: Pestiviruses (bovine diarrhea), Flaviviruses (dengue and yellow fever), and the Hepacivirus genus in which hepatitis C and G are included (Bradley & Maynard 1986).

HCV genome organization, viral proteins and genotypes.
The RNA genome of HCV consists of a single uninterrupted open-reading frame, approximately 9500 nucleotides in length, bracketed by 5'- and 3'-non-coding regions (NCR), highly conserved. Protein translation begins at an internal ribosomal entry site in the 5'-NCR. The HCV open reading frame (ORF) encodes a precursor polyprotein of approximately 3000 amino acids, which undergoes post-translational cleavage by host and viral proteases to produce a series of viral proteins. Among the structural proteins there are core proteins (C) and envelope proteins (E1 and E2) that are located at the N-terminal region of the polyprotein precursor, while nonstructural (NS) proteins include NS1, NS2, NS3, NS4 (A and B), NS5 (A and B) located at the C-terminus (Graikou et al. 1993).

The non-structural regions have a role in virion replication and enzyme encoding: the NS1 region encodes p7 protein; the NS2 region encodes transmembrane protein; the NS3 region encodes metalloprotease, serine protease, RNA helicase; the NS4 regions (A and B) are responsible for the NS3 protease cofactor; the NS5A region encodes p56 and p58 related to the IFN-resistance protein; and NS5B encodes p68, the RNA polymerase protein.

The function of the NS5A protein has not been defined, although it is assumed to be important for RNA replication. Studies have indicated that mutations in this region may be related to IFN responsiveness (Enomoto et al. 1996), and NS5A may promote IFN resistance by repressing IFN-induced double-stranded RNA-dependent protein kinase (PKR) (Gale et al. 1997).

Figure 1: RNA genome organization and encoded proteins of hepatitis C virus (HCV)
Among structural proteins there are the p22 (core protein) that is thought to interact with RNA to form the virion nucleocapsid (Yasui et al. 1996) and gp35 (E1) and gp70 (E2) interaction is responsible for the putative HCV envelope protein complex. As NS5A products, gp70 (E2) may also influence IFN responses. Actually, there are high degrees of variation in the amino acid sequences in the N-terminus of the E2 protein. This region is referred to as the hypervariable region 1 (Major & Feinstone 1997). This region is probably presented on the surface of the virion as a part of the viral envelope, and contains a neutralizing epitope, which is susceptible to immune pressure and the selection of escape mutants.

![Diagram of hepatitis C virus (HCV)](image)

**Figure 2: Model structure of hepatitis C virus (HCV)**

Comparison of HCV cDNA nucleic acid sequences has led to a better understanding of the variation of HCV RNA genome. Variations as high as 34% occur among the most divergent strains. Based on the observed variations, HCV can be further classified into different genotypes and subgenotypes. On the 5’NCR region are defined genotypes and subgenotypes from HCV. Genotypes are defined as major branches in the HCV phylogenetic tree, whereas subgenotypes represent more closely related sequences within a major genotype. Six different HCV genotypes have been identified and designated as types 1-6. Subgenotypes are usually designated by letters such as a, b or c (Simmonds 1995).

The virion half-life is between 3 and 5 h, with a clearance and production rate of approximately $10^{12}$ particles per day, corresponding to 50 particles per infected hepatocyte per day (Neumann et al. 1998).

The prevalent HCV genotypes vary in different geographical regions. Genotype 1a predominates in USA, type 1b and 2a are most common in Japan, genotype 4 is restricted to the Middle East and Central Africa, genotype 5 to South Africa and genotype 6 in Hong Kong (Freeman et al. 2001). Several studies have been conducted to determine the distribution of HCV genotypes among different groups of individuals in Brazil. Most of these studies indicate a higher prevalence of genotype 1, followed by genotypes 3 and 2. (Gonçalves et al. 1993, Busek & Oliveira 2003).

It has been shown that hepatitis C virus is associated with low-density lipoproteins (LDL) in human sera. An interaction between HCV–LDL complexes and the LDL receptor may be responsible for HCV binding to and crossing through the cell membrane. However, the specific mechanism by which HCV particles interact with the LDL receptor prior to internalization remains unclear. In addition, HCV-E2 protein has been shown to bind the major extracellular loop of human CD81 protein, a cell surface molecule, expressed on virtually all nucleated cells (Agnello et al. 1999, Monazahian et al. 1999, Pileri et al. 1998, Wünschmann et al. 2000). However, recent evidence suggests that this interaction is not important for HCV entry into cells such as hepatocytes (Meola et al. 2000). Hepatitis C virus envelope proteins may be important for fusion with the endosomal membrane through binding alternative, unidentified, cell surface proteins (Takikawa et al. 2000).

**Diagnosis.**

Diagnostic tests commercially available today are based on Enzyme immunoassorbent assays (EIA) for the detection of HCV specific antibodies. EIAs can detect more than 95% of chronically infected patients but can detect only 50% to 70% of acute infections. A recombinant immunoblot assay (RIBA) that identifies antibodies, which react with individual HCV antigens, is often used as a supplemental test for confirmation of a positive EIA result. Testing for circulating HCV by RNA amplification tests (e.g. polymerase chain reaction or PCR, branched DNA assay) is also being utilized for confirmation of serological results as well as for assessing the effectiveness of antiviral therapy. A positive result indicates the presence of active infection and a potential for spread of the infection and or/the development of chronic liver disease (WHO 2002).
Immune response to HCV.

In general, when viral infection occurs, NK cells are activated and, nonspecifically recognize cells undergoing changes caused by infection and act to kill them. Additionally, infected cells produce interferon (IFN) α/β which leads to suppression of viral replication. In the next stage specific responses arise where antibodies bind to viral particles in body fluids, neutralizing and eliminating them by either complement or antibody-dependent cellular cytotoxicity. Cytotoxic T cells (CD8+) are generated that kill infected cells using two major mechanisms: the performing granule exocytosis pathway and FasL/Fas mechanism of apoptosis. This process ends virus infection.

The number of NK cells in human liver is higher than in peripheral blood or any other organ (Doherty et al. 1999). They can display stimulatory or as well as inhibitory receptors and may distinguish infected from uninfected cells by recognizing alterations in MHC class I expression or changes in cell-surface glycoproteins induced by viral infection. In the early stage of infection NK cells are activated by IFN-γ and by IL-12 produced by macrophages and dendritic cells. NK cells release granules into target cells leading them to apoptosis. NK cells also produce cytokines, such as IFN-γ and tumor necrosis factor (TNF)-α, and a variety of other chemokines, such as macrophage inflammatory protein (MIP) 1α/1β.

The production of antibodies and cytotoxic cells is controlled by helper T cells (CD4+) that are activated when they recognize viral fragments bound to glycoproteins called major histocompatibility complex (MHC) class II molecules on the surface of antigen presenting cells (APC), such as dendritic cells, macrophages and B cells. Type 1 helper T (Th1) cells produce interleukin (IL) 2, IFN-γ and TNF-α, promoting activation and proliferation of CD8+ cells and NK cells. Type 2 helper T (Th2) cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13, which promote B-cell differentiation into antibody-producing plasma cells. The action of CD8+ cells is by recognition of peptide viral fragments bound to cell surface glycoproteins called MHC class I molecules. APC produce IL-12 that improves Th1, CD8+, and NK cells. IL-10 also acts on APC suppressing IL-12 production and its active on Th1, leading to termination of cellular immune response to the virus.

It is assumed that the HCV-virus-specific immune response is primed in the regional lymph nodes where APC present viral antigen peptides on MHC class II molecules to CD4+ T cells and, via cross-priming on MHC class I, to CD8+ T cells. Activated HCV-specific T cells leave lymph nodes as effector cells through efferent lymphatic vessels, circulate in the blood, and enter the liver via the large portal vein and the small hepatic artery ( Mizukoshi & Rehermann 2001).

Patients with self-limited HCV infection show more vigorous peripheral blood proliferative CD4+ cell responses to HCV-derived proteins (NS3, NS4 or NS5) and patients with chronic infection show weaker responses. Healthy status was also linked with the presence of T-cell response to HCV core protein and to HCV non-structural proteins. In the chronic state, besides the high degree of activation, the proportion of HCV antigen-specific cytotoxic T cells in liver is very low, a least for the epitopes used in tetramer assays (Valantine et al. 2000).

The role of the HLA system.

In the human major histocompatibility complexes (MHC) there are three main class I a chain loci, called HLA-A, -B and -C and three pairs of class II α and β chain loci, called HLA-DR, -DP and -DQ. In addition, there are several genes, such as complement components C2, Factor B, and C4, TNF-α and TNF-β, called class III genes mapped within MHC with important functions in immunity.

The class I molecules include the gene products of HLA-X, HLA-E, HLA-J, HLA-H, HLA-G, and HLA-F loci and they are characterized by a restricted tissue distribution and by limited polymorphism. Class I molecules also include the family of stress-induced MHC class I chain-related molecules (MICA and MICB) that are related to NK and CD8+ T lymphocyte activity.

The class II region of MHC also contains two other genes called Transporter Antigen Processing (TAP) 1 and 2. These genes encode heterodimer protein that is important to transport peptides from the cytosol into endoplasmic reticulum, where peptides can be associated with newly translated class I MHC α-chain.

Exogenous virus proteins are taken up into intracellular vesicles of APC cells, where they are degraded into peptide fragments and bind to MHC class II molecules. Endogenous virus proteins, produced in the cytosol of infected cells, are degraded to peptide fragments by the proteasome (LMP2/7 MHC-genes), and the fragments are actively transported from the cytosol into the endoplasmic
reticulum by TAP 1 and 2 heterodimer proteins. Subsequently, they are bound by MHC class I molecules, and the complexes are transported through the Golgi apparatus to the cell surface.

![Diagram of human major histocompatibility complex](image)

**Figure 3**: The arrangement of genes within the human major histocompatibility complex.

A genetically determined resistance or susceptibility to chronic hepatitis C virus infection may make an important contribution to the course of liver disease and may be linked to the human MHC. The involvement of different genes in various HLA sub-regions suggests that anti-HCV responses are modulated by complex gene interplay rather than by single alleles.

Cytokines are soluble hormone-like proteins associated with inflammation, immune responses, tissue injury, repair, and organ dysfunction. IL-10 and TNF-α genes have a number of polymorphisms in the promoter region which influence the level of cytokine secretion. While a high level of TNF-α is associated with spontaneous HCV elimination, IL-10 level has no effect on HCV elimination and a high level may be counter-productive (Thursz 2001). In fact, two cytokine patterns were observed. Self-limited infection is related to moderate levels of IL-2, high levels of INF-γ and little or no IL-10 (Th1 type response), and in persistent viremia is related to lack of IL-2, low levels of INF-γ and high secretion of IL-10 (Th2 type response) (Eckels et al. 2000).

CD4+ T cell clones prepared from liver tissue of patients with chronic hepatitis C produced Th1 cytokine following stimulation with recombinant HCV protein (Napoli et al. 1996). This finding suggests that intrahepatic secretion of Th1 cytokine likely stimulates CD8+ T cell response. Although lower expression of IFN-γ and IL-4 has been observed in patients who failed to respond to IFN therapy increased expression of IL-4, IL-10 and TGF-β1 and/or exhaustion of IFN-γ has also been reported in these patients. Patients with genetical predisposition to high IL-10 production, as determined by heterogeneity in the promoter region of the IL-10 gene, have a poor initial IFN response (Edwards-Smith et al. 1999). However, that finding was complicated by other clinical parameters, making the interpretation difficult.

The predictive value of hepatic IFN receptor gene expression for the IFN response has recently been assessed. These studies indicated that a higher level of hepatic IFN receptor mRNA was associated with a more favorable IFN response. This conclusion is supported by an immunohistochemical study showing the increase of IFN α/β receptor expression in the hepatocyte, among IFN well-responder patients (Yatsuhashi et al. 1999). Correspondingly, increased expression of IFN receptor mRNA and IFN response was also associated with a lower HCV level (Mathai et al. 1999).

IFN-γ causes an increase in HLA class I expression and induction of HLA class II antigens on endothelial and bile duct epithelial cells. In the case of hepatocytes there are significantly less HLA class I and induction of HLA class II antigens after IFN-γ. This may explain why T-lymphocytic infiltrates are found predominantly in portal fields in chronic status, and they are main represented by cytotoxic T cells with specificity for HCV core protein (Schroder et al. 1995).
Using limiting dilution assay, an increase of precursor helper T cells was found in peripheral blood, and presence of cytotoxic T cells, in liver of those patients with maintenance of the non-detection-state of HCV-RNA after IFN treatment. These individuals are known as IFN-sustained response patients (Lohr et al. 1999).

When other kinds of assay are applied, such as lymphocyte proliferation, using synthetic peptides from HCV proteins, controversial results have appeared. Some authors have reported that IFN-α-therapy efficiency is related to T-cell response against core and envelope (E1 and E2) proteins, but not against NS3, non-structural protein (Leroux-Roels et al. 1996). For others, T-cell responses to core and NS5 proteins were associated with viral persistence, while NS3 and NS4 T-cell responses were associated with viral clearance (Lohr et al. 1998). The HCV viral load level may also influence the T (helper or cytotoxic) cell responses increased by IFN-α treatment (Lohr et al. 1999).

The role of the host’s humoral immune response in providing protection against HCV infection is questionable. The relatively low level and delayed appearance of HCV-specific antibodies in most patients with HCV infection, suggest that the humoral immune response may play a relatively minor role in HCV clearance, and this point remains controversial. It has been reported that anti-HCV antibodies failed to protect rechallenge of a convalescent chimpanzee with homologous or heterologous HCV strains (Farci et al. 1992) and, on the other hand, that pooled human sera containing polyclonal antibodies to HCV may protect chimpanzees from HCV infection (Feray et al. 1996, Krawczynski et al. 1996, Yu et al. 2004).

The role of antibodies during HCV infection was determined using a neutralizing binding assay, which evaluates antibody capacity on binding-inhibition of HCV-E2 protein to human cell receptors. The maintenance of high-level antibody in chronic hepatitis C is correlated with spontaneous resolution. This point contributes to the importance of the humoral response in HCV clearance. Nevertheless the HCV protein antibody specificities may be most important. It has been reported that anti-hypervariable region 1 (HVR1) antibodies in patients infected with HCV-1b genotype, the broad reactivity of serum anti-HVR1 antibodies is correlated with higher viral loads and lower IFN-α response rate (Hattori et al. 1998).

Studies of HLA-disease associations depend largely on patient geography and ethnicity and have shown inconsistent results. However, any relevant association with HCV disease progression may be helpful to understand its mechanism. So, the liver disease severity and HCV elimination (or persistence) may be under the influence of HLA molecules. There are certain HLA class II genes with consistent correlation with infection resolution. The protective class II molecule may present particular peptides, which are both, highly conserved and well expressed, processed and presented to helper T cells.

Several studies have revealed an association between HLA type and protection against chronic HCV infection. They demonstrated that HLA-DRB1*01, -DRB1*04, -DRB1*1101/04 (DR5), -DQA1*03, -DQB1*0301/02 are associated with spontaneous HCV elimination and are reduced in patients with chronic disease. The primary HCV clearance--HLA-DR association seems likely to be with HLA-DQB1*0301 and the associations with HLA-DRB1*04 and HLA-DQA1*03 may be due to linkage disequilibrium (Alric et al. 1997, Cramp et al., 1998, Minton et al. 1998, Tilbs et al. 1996).

Though there is a consensus that the HLA-DQB1*0301 allele is important in HCV spontaneous clearance, this association is not universal. For instance, a strong association was observed between this allele and clearance of HCV in black subjects and weakly associated in white ones. In white subjects, viral clearance was associated with HLA-DRB1*0101 and its HLA-DQB1*0501 haplotype, whereas viral persistence was associated with HLA-DRB1*0301 and its HLA-DQB1*0201 haplotype (Thio et al. 2001) and with HLA DRB1*0301 and DRB1*1301/2 alleles (Hohler et al. 1997). HLA-B54 antigen was associated with liver injury progression (Kuzushita et al. 1998). It appears that other factors, including age, sex and HCV genotype are significantly more important than HLA alleles in correlating with the severity of the disease. Additionally, racial differences may be responsible for conflicting data on HLA association with hepatitis C outcome.

Other protected associations were found such as HLA-DR13 antigen that means to protects the newborn from vertical HCV transmission (Bosi et al. 2002).

Class II HLA genes may be also involved in chronic HCV infection severity. HLA-DR1*03 and -DQB1*0201; -DQB1*0502; -DQB1*0503 allele
frequencies were increased in patients with liver cirrhosis, while HLA-DRB1*11 and -DQB1*03 alleles were decreased. HLA-DR3 plus HLA-B8 antigens were associated with fibrosis and with hepatocellular carcinoma (Hue et al. 2002, Lopez-Vazquez et al. 2004, Mangia et al. 1999, Tillmann et al. 2001).

The relationship between HLA haplotype, natural history of disease and interferon response has also been assessed. However, the results remain controversial and inconclusive. For instance, after interferon-alpha treatment, sustained response was associated, in Taiwanese with HLA-A11, -B51, -Cw15, -DRB1*15 and DQB1*05; in Canadians with HLA-DRB1*0404; in Poles with HLA-DRB1*0701 -DQA1*0201 -DQB1*02, whereas no interferon-alpha effect was associated with HLA-A24 among Taiwanese patients, HLA-DRB1*07 among French patients or HLA-DRB1*07 -DQB1*02, in Brazilian patients (Alicic et al. 1999, Corghi 2005, Sim et al. 1998, Wawrzynowicz-Syczewska et al. 2000, Yu et al. 2003).

As CD8+ T cells are the major effectors and recognize antigenic peptides in the context of class I MHC, the HLA class I molecule itself may also be important to HCV infection outcome. Patients expressing HLA molecules incapable of presenting early or early-immediate viral protein may not be able to mount a response to the first wave of infection. Not all virus epitopes are equal in their capacity for activating T cells. Some epitopes (immunodominant) elicit vigorous polyclonal T cell responses in a majority of infected individuals, whereas others (subdominant) are poorly immunogenic, and some (cryptic) may only be revealed by peptide stimulation in vitro. Maximal and prolonged stimulation by immunodominant epitopes may result in T cell exhaustion and leads to unresponsiveness or death by apoptosis. Apoptosis is a physiological pathway of programmed cell death, and it is a highly conserved evolutionary process for deleting senescent, damaged, redundant or deleterious cells. It was been well documented that apoptosis plays a key role in liver injury during HCV infection. HCV core protein may enhance both Fas-mediated and tumor necrosis factor (TNF)-induced apoptosis (Ruggieri et al. 1997, Zhu et al. 1998). Thus, HCV core protein may promote cell death during HCV infection through various signaling pathways. However, the core protein has also been shown to inhibit TNF-α-induced apoptotic cell death (Ray et al. 1998). The remaining specific T cells are able to control viral replication but incapable of eliminating infection. So, the patient progresses to a chronic infective state. As an example, HCV core protein contains only one HLA-B7 epitope (Rosenberg 1999). On the other hand, HLA-B44-positive patients have significantly higher cytotoxic T cell activity of several HCV epitopes (Hiroishi et al. 1997).

HLA-A*03, HLA-B*27, and HLA-Cw*01 are more frequently encountered in patients with viral clearance compared to those with chronic infection. HLA-B*08 and HLA-Cw*04 occur more often in those with chronic infection compared with viral clearance (McKieman et al. 2004, Thio et al. 2002).

It has been suggested that antigen-specific T cell activation requires both presentation of antigen by MHC molecules and the delivery of co-stimulatory signals. An in vitro assay showed that the endogenous core protein was presented to helper T cells, through HLA class II along with B7/BB-1, one of the most important accessory molecules, that induces the co-stimulatory signals (Chen et al. 1998).

The HCV protein can also up-regulate class I MHC, a surface molecule, by TAP1 gene expression interference. As a consequence of increased MHC class I expression, there will be a significantly down-regulated cytotoxic activity of NK cells (Herzer et al. 2003). However, other authors reported that functional MHC class I cell-surface expression and intracellular proteasome activity were not affected by the expression of HCV proteins (Moradpour et al. 2001).

The role of nonclassical MHC class I chain-related A (MICA) genes has been investigated. Its protein acts as a ligand to NKG2D, a stimulatory receptor present on macrophage, CD8+ T cells, gamma-delta T cells, NKT cells and natural killer NK cells. MICA is highly polymorphic, and there is evidence that the various allotypes bind to NKG2D with different affinities. Actually MICA*15 was detected more than two-fold more often in persons with viral clearance, and it occurred in fewer than 5% of persons with persistent hepatitis C (Karacki et al. 2004).

**Liver injury**

It is not clear whether HCV directly causes liver damage or whether this is a consequence of the host's immune response. Antibodies and cytotoxic T cells depend upon appropriate activation...
of helper T cells and they are required for successful eradication of HCV. On the other hand, the level of IL-2, IFN-γ (Th1 profile cytokines) correlates with the damage observed in chronic liver disease caused by long-term HCV infection in the majority of patients (Napoli et al. 1998). Actually, patients with chronic HCV have shown elevated levels of serum IL-4 and IL-10 (Th2 profile cytokines) as well as IL-2 and IFN-γ cytokines (Cacciarelli et al. 1996) although in vitro, virus antigens induce preferentially Th1 cytokine profiles.

Some authors had reported the presence of lymphoid infiltration without cytopathic changes in hepatocytes (Scheuer et al. 1996). The lymphoid infiltrate contains a predominance of CD8+ T cells (cytotoxic) over CD4+ T cells (helper). While CD4+ T cells are confined to the portal and periportal areas, CD8+ T cells contribute to the lobular infiltrate (Fiore et al. 1997). Also, the presence of CD8+ T cells in contact with apoptotic bodies and hepatocytes containing HCV antigens has been demonstrated (Krawczynski et al. 1992). The presence of non-specific cytotoxic cells (NK) recruited by IFN-γ and viral hepatitis E2-specific antibodies involved in antibody-dependent cellular cytotoxicity against infected host cells may contribute to hepatocellular damage (Bertoletti & Maini 2000).

There is some evidence that HCV also infects peripheral blood mononuclear cells (PBMC) in addition to hepatocytes. CD81 molecules have been identified as E2-HCV-protein receptors into B lymphocytes (as well as monocytes) and may provide a repository of virus that may continually re-infect the liver, despite the Th1 type immune process in liver. In T cells the E2 interaction results in a co-stimulation signal, but in NK cells leads to a suppression of cytotoxic activity (Bartosch et al. 2003, Eckels et al. 2000).

**HCV immune response evasion.**

In order to cause chronic infection, the virus must evade host immune responses. In acute HCV infection a vigorous antiviral T lymphocyte response is associated with viral clearance. In chronic HCV specific T lymphocytes are weak or absent. It has been argued that HCV must have developed a means either to not induce or to actively antagonize immune responses. In the course of infection HCV subverts the command and control center of the immune system, by evolving epitopes that down regulate antiviral Th1 responses and up regulate Th2 cytokines, which foster host tolerance to HCV.

HCV is thought to be a non-cytopathic virus and this can contribute to generating a poor immune response. The amount of exogenous (out of cell) antigens that can be taken up by dendritic cells will be scarce. Internalization and reprocessing of exogenous antigen by APC are important to activate CD4+ T cells.

Beside this, the HCV genome exhibits significant genetic heterogeneity as a result of accumulation of mutations during viral replication. So the mechanism of HVC persistence also depends on its quasispecies nature. More than 20 strains of HCV have been cloned from a single patient at a single time (Farcì et al. 2000). The hypervariable region 1 (HVR1) of the E2 envelope gene is mainly responsible for significant inter- and intra-individual variation of HCV. Although cross-reactivity for unrelated HVR1 peptides exists in the majority of the patients (Mondelli et al. 1999), some patients have highly strain-specific antibodies and are incapable of preventing the emergence of viral variants. (Peoples et al. 2000). When the immune system is confronted by previous and new HCV epitope variants, instead of generating a fresh response to the new variant, the host responds with antibodies and T cells previous elicited by the original wild type (Rosenberg 1999).

Viral variants can also escape from T cell recognition. Recognition of antigens by T lymphocytes requires both binding of antigenic peptides by MHC molecules and interaction of MHC-peptide complexes with T cell receptors (TCR). Mutations at anchor residues may lead to a loss of MHC binding and thus a failure of antigen presentation. Mutation that occurs at TCR contact residues, or those influencing the conformation of TCR contact residues, may destroy T cell recognition and thus prevent T cell activation. Other kinds of mutation can subtly alter the virus peptide, that still binds to the same MHC molecule and engages the same antigen specific TCR, but leads to T cell unresponsiveness (Rosenberg 1999). So the antigen processing in APC cells might also result in non-immunogenic peptide fragments that can modify T cell activation. The loss of an immunodominant epitope by the virus may be sufficient to establish persistent infection.

**Treatment.**

Interferons (IFN) are proteins that can modu-
late biological responses. They belong to three families: alpha, beta and gamma. Only α- and β-interferon, collectively known as class I interferons, are effective against HCV, and β-interferon offers no advantage over recombinant human α-interferons for most populations. The precise mechanism of action has not been determined for IFN α products. IFN α has been shown to induce antiviral and immunomodulatory actions in stimulated cells.

Interferon taken alone or in combination with ribavirin (RBV), a synthetic guanosine analogue, has been used for the treatment of persons with chronic hepatitis C. Treatment with interferon alone is effective in about 10% to 20% of patients. Interferon combined with ribavirin is effective in about 30% to 50% of patients. Ribavirin does not appear to be effective when used alone (WHO 1999).

A new interferon, denominated PEG-interferon, has been developed and this improves the efficacy of α-interferon against chronic HCV. Pegylation is a process by which an inert molecule of polyethylene glycol is covalently attached to a protein, giving it a higher molecular weight. This slows the rate of absorption from subcutaneous sites and impairs pathways of protein degradation, thereby greatly prolonging the serum half-life and prolonging the biological effects of the protein.

PEG-IFN α has been shown to be more effective with or without RBV in the management of treatment of naive patients with chronic genotype 1 HCV infection, than the unmodified IFN. For the more responsive genotypes the sustained virological response is between 75% and 85% (Teoh et al. 2004).

A virological response in chronic HCV is defined by a reduction of HCV-RNA in serum and ideally by absence of detectable HCV-RNA by qualitative reverse transcriptase-polymerase chain reaction assay with a sensitivity of approximately 100 copies (50 IU) per mL. The sustained virological response to antiviral treatment of chronic HCV has several beneficial effects. These include improved liver histology with arrested progression of hepatic fibrosis and fibrotic regression, substantially reduced risk of hepatocarcinoma, decreased rates of hepatic failure, liver transplantation and liver-related death. PEG-IFN in combination with RBV is now the first line treatment for chronic HCV, but the cost of treatment is still very high.

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