Review Article

Immunopathogenesis of hepatitis C virus infection

ANTHONY J FREEMAN, GEORGE MARINOS, ROSEMARY A FFRENCH and ANDREW R LLOYD

Viral Hepatitis Research, Gastrointestinal and Liver Unit, The Prince of Wales Hospital, 3Westfield Research Laboratories, Sydney Children’s Hospital, Randwick and 2Inflammation Research Unit, School of Pathology, The University of New South Wales, Kensington, New South Wales, Australia

Summary Hepatitis C virus, a recently identified member of the family Flaviviridae, is an important cause of chronic viral hepatitis and cirrhosis. There are similarities in the nature of the immune response to this pathogen with immunity in other flavivirus and hepatotropic virus infections, such as hepatitis B. However, the high rate of viral persistence after primary hepatitis C infection, and the observation that neutralizing antibodies are not protective, would suggest that there are a number of important differences between hepatitis C, other flaviviruses, and hepatitis B. The phenomenon of quasispecies evolution and other viral factors have been proposed to contribute to immune evasion by hepatitis C virus. In the face of established persistent infection, virus-specific cytotoxic T lymphocytes may exert some control over viral replication. However, these same effectors may also be responsible for the progressive liver damage characteristic of chronic hepatitis C infection. The nature of protective immunity, including the role of innate immune responses early after hepatitis C exposure, remains to be defined.

Key words: CD8, chronic hepatitis, cytotoxic T lymphocytes, Flaviviridae, hepatitis C virus, immunity, liver, viral hepatitis, viral persistence.

Hepatitis C pathogenesis remains an important scientific challenge 10 years after the discovery of the virus

Hepatitis C virus (HCV) is a significant public health problem. Approximately 3% of the world’s population has persistent HCV infection.1 In 1989, the virus was identified as the major aetiological agent responsible for post-transfusion non-A and non-B hepatitis.2–6 Following primary HCV infection, persistent viraemia and chronic hepatitis develop in the majority of cases.7 Patients are at risk of progressive hepatic fibrosis, cirrhosis and death from liver failure, as well as the advent of hepatocellular carcinoma.8 Efforts to elucidate the mechanisms behind viral persistence and hepatocellular damage have been frustrated by the lack of a reliable cell culture system for viral propagation in vitro. In addition, as the chimpanzee is the only experimental animal susceptible to HCV infection, progress in research is hampered by the lack of a small animal model to facilitate pathophysiological studies as well as the evaluation of antiviral treatment and vaccine strategies.

The epidemiology and clinical aspects of HCV infection are briefly outlined below, followed by a summary of aspects of the molecular virology of HCV. The review of immunity in HCV infection focuses on: (i) the role of viral diversity in immune evasion, and other potential mechanisms of HCV persistence; (ii) the contribution of immune responses to hepatocellular injury; (iii) studies investigating potential components of protective immunity against HCV, notably HCV-specific cytotoxic T lymphocytes (CTL); and (iv) the unique immunological environment encountered in the liver. The pathogenesis of HCV-related fibrosis, hepatocellular carcinoma and autoimmune disease associated with HCV infection are not discussed in detail. Potential insights into the immunopathogenesis of HCV infection may be gained by considering the immune response to other members of the Flaviviridae family, and to the hepatitis B virus (HBV), another viral agent responsible for chronic liver disease.

Hepatitis C is an hepatotropic flavivirus

Hepatitis C virus was cloned from the plasma of an experimentally infected chimpanzee and characterized as a positive sense, ssRNA virus.2 On the basis of genome homology and hydrophobicity patterns, which are similar to the pestiviruses and flaviviruses, HCV has been classified as the first member of a third genus called Hepacivirus within the family Flaviviridae.9,10 Pestiviruses, such as bovine viral diarrhoea virus (BVDV), are important animal pathogens. In contrast, flaviviruses are responsible for disease in humans: including acute encephalitic infections (e.g. Japanese B encephalitis); febrile syndromes with rash, exemplified by dengue; and viral haemorrhagic fevers, such as yellow fever.11 The latter two diseases may include acute hepatitis as a feature. Other than with HCV, persistent infection is rare after primary flavivirus infection. Successful flavivirus vaccines have been developed, including the live, attenuated yellow fever vaccine, and the formalin-inactivated Japanese B encephalitis vaccine.12 However, there is currently no effective immunization strategy for the prevention, or treatment, of HCV infection.
Similar to HCV, HBV is an hepatotropic virus that can cause persistent infection, chronic hepatitis, cirrhosis and hepatocellular carcinoma. Hepatitis B virus is a non-cytopathic virus with a small, circular dsDNA genome and a lipoprotein envelope. In contrast to HCV, 95% of adults with primary HBV infection experience a self-limited illness that is attributed to an effective immune response and viral clearance. Vaccination with hepatitis B surface antigen results in the generation of protective antibody and a long-lived anamnestic response in the majority of cases.

**Hepatitis C is a common pathogen**

Hepatitis C infection has been documented in sera stored in the 1950s. To the end of 1997, 190,000 Australians (i.e. approximately 1% of the population) were estimated to have antibodies to HCV and the annual incidence of new infections was estimated at 11,000. In the United States approximately 2.7 million persons suffer from persistent HCV infection. The prevalence is considerably higher in developing countries, such as parts of Africa and the Middle East. Hepatitis C virus represents a major economic burden to the health-care system because of the substantial medical costs incurred in managing patients with persistent infection.

**Hepatitis C is spread through blood-to-blood exposure**

Hepatitis C virus transmission occurs principally through parenteral exposure to contaminated blood or blood products. Because of the introduction of blood product and organ donor screening (in 1990 in Australia) the main risk factor for HCV infection remains injecting drug use. Between 50% and 70% of Australian injecting drug users are infected with HCV. Other risk factors for HCV infection include acupuncture, tattooing, haemodialysis, occupational exposure in health-care workers, and the re-use of needles in mass vaccination programs. The rate of transmission from mother-to-infant is approximately 6%. The majority of vertical HCV transmission occurs at the time of delivery. Early studies suggest that transmission is more common in the setting of high maternal viral load or in the presence of concomitant HIV infection. However, a recent study demonstrated that the viral load was the same in transmitting and non-transmitting women, and that vertical transmission was associated with the presence of negative-strand intermediates in maternal peripheral blood mononuclear cells. This supports the hypothesis that the transfer of infected cells to the infant is the major cause of vertical HCV transmission. Breast-feeding is not thought to increase infection risk to babies. Hepatitis C virus is not found consistently in bodily secretions, thus sexual transmission is rare. A small proportion of patients with no identifiable risk factor for hepatitis C are designated as sporadic cases and presumably reflect unrecognized or unreported blood-to-blood exposure. The majority of flaviviruses are transmitted to vertebrates by infected arthropods. However, mosquitoes fed HCV-infected blood are only transiently positive for viral RNA and are, therefore, incompetent as a vector of HCV.

The liver in chronic hepatitis C contains a lymphocytic infiltrate and develops progressive fibrosis

The histological features of persistent HCV infection are those of chronic hepatitis, with expanded portal tracts and lobular inflammation of varying severity. The infiltrate contains predominantly CD8+ T cells (Fig. 1). Lymphocytes may form focal aggregates that occasionally take the form of follicles with germinal centres. The limiting plates of hepatocytes around portal tracts may be eroded by interface hepatitis, also known as piecemeal necrosis. In contrast to chronic hepatitis B, confluent ‘bridging’ necrosis is uncommon. Hepatocytes in chronic HCV hepatitis may show acidophilic degeneration within intact plates and form apoptotic (Councilman) bodies. Bile duct damage and proliferation may also occur. Microvesicular fat accumulation (steatosis) is common, but rarely severe. Iron deposition (siderosis) is also rarely severe. However, in patients with thalassaemia major who receive multiple blood transfusions, or those with the inherited iron storage disorder, haemochromatosis, HCV can act synergistically with iron to promote liver damage. Progressive fibrosis occurs in patients with persistent HCV infection with a generally increasing prevalence over decades of chronic hepatitis. Once cirrhosis has developed the histological changes are less characteristic.

Liver biopsies in patients with chronic hepatitis C hepatitis are generally evaluated in terms of grade and stage. Grading is used to describe the intensity of necro-inflammatory activity. Staging is a measure of the fibrosis and architectural distortion that are the precursors of cirrhosis. A numerical score, for example the Knodell score with modifications by Ishak is commonly assigned to both grade and stage, thereby providing a semiquantitative measurement of the observed histological features.

Primary hepatitis C virus infection results in persistent infection and cirrhosis in a significant proportion of cases

Primary HCV infection is predominantly asymptomatic and fulminant hepatic failure in this context is rare. Serum HCV RNA can be identified by PCR as early as 7–21 days after exposure. There is a decline in the level of viraemia around the time of initial alteration in biochemical tests of liver function, notably a rise in the level of serum transaminases. Seroconversion typically occurs after approximately 2–8 weeks. Patients who present with jaundice (thus indicating more severe liver injury) during primary infection are more likely to clear HCV than the majority who acquire HCV without apparent symptoms. Some estimates suggest that persistent infection develops in approximately 80% of cases of HCV. However, considering that primary infection is predominantly asymptomatic and that antibody disappears in the intermediate term in a proportion of those who clear the virus, the true figure may be closer to 50%. The majority of patients with persistent HCV will have ongoing biochemical and histological evidence of hepatitis, but are typically either asymptomatic or have only mild fatigue. The most readily available marker of liver injury, the serum transaminase levels, characteristically fluctuate widely over time, correlate poorly with histological
disease activity and may be normal in patients with severe hepatitis and cirrhosis.\textsuperscript{59–61}

The major morbidity and mortality of persistent HCV infection occurs after the establishment of hepatic fibrosis and the subsequent occurrence of cirrhosis and hepatocellular carcinoma.\textsuperscript{8} In those patients who develop clinically apparent HCV-related liver disease, the evolution is typically very slow with an average time to presentation with chronic hepatitis, cirrhosis and hepatocellular carcinoma of approximately 10, 20 and 30 years, respectively.\textsuperscript{62} As the infection is typically asymptomatic in the majority of patients, and runs a protracted course, it has been difficult to define the true proportion of cases that will progress to cirrhosis.\textsuperscript{63} There have been a large number of published series of patients recruited through specialist clinics that report an HCV-related cirrhosis rate in excess of 30\% after 20 years infection.\textsuperscript{64,65} These cross-sectional series of referred patients, while containing large numbers of subjects, give a distorted picture of the natural history of HCV infection due to a selection bias towards patients with more severe disease. Data from population-based cohorts suggest that less than 10\% of patients with persistent HCV infection, acquired in young adulthood, will progress to cirrhosis after 20 years infection.\textsuperscript{66} The situation in the third and fourth decades is less certain, however, there is evidence that the rate of progression of hepatic fibrosis beyond 20 years is linear.\textsuperscript{43} Consistent with this impression of a more favourable outcome, a recent 45 year follow up of 17 American male military recruits with persistent HCV infection demonstrated that only 2 (12\%) had developed advanced liver disease.\textsuperscript{17} Factors shown to accelerate progression to cirrhosis in persistent HCV infection include older age at HCV infection, male gender, heavy alcohol intake and co-infection with either HBV or HIV.\textsuperscript{43,67–69} The annual incidence of hepatocellular carcinoma is approximately 3\% among HCV positive patients with cirrhosis.\textsuperscript{70} With regard to mortality, subjects with post-transfusion HCV infection followed-up 25 years after acquiring HCV during open-heart surgery had no excess all-cause mortality.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Chronic hepatitis C virus (HCV) infection is characterized by a lymphocytic infiltrate. (a) Uninfected human liver, demonstrating a portal tract (PT) surrounded by hepatocytes (H) stained with haematoxylin and eosin (H&E). (b) Chronic HCV infected liver with an expanded portal tract (PT), interface hepatitis (IH) and lobular inflammation (LI) (H&E). (c) Chronic HCV infected liver stained with anti-CD3 antibody to detect T lymphocytes. (d) Chronic HCV infected liver stained with anti-CD8 antibody. Arrows identify antigen positive cells. Reproduced with kind permission of CE Harvey and JJ Post. Magnification ×400.}
\end{figure}
mortality and only a small increase in liver-related mortality compared with age-matched controls.\textsuperscript{71,72} The mortality attributable to community acquired HCV infection as a result of injecting drug use remains to be defined.\textsuperscript{73}

Current therapies for hepatitis C are only partially effective

The goal of treatment is to eradicate HCV and prevent progression of liver disease. The two drugs currently available are IFN-\(\alpha\), a cytokine with immunomodulatory and antiviral activity, and ribavirin, a synthetic guanosine nucleoside analogue with \textit{in vitro} antiviral activity.\textsuperscript{74} After a 48-week course of parenteral IFN-\(\alpha\) monotherapy, less than 20\% of patients with persistent HCV infection will clear the virus and show a sustained normalization of serum transaminase levels.\textsuperscript{75,76} A four-year follow up of treatment responders suggests that patients with persistently undetectable HCV RNA are cured.\textsuperscript{77} Ribavirin monotherapy has been shown to transiently decrease serum transaminase concentrations, but has no effect on serum viral load.\textsuperscript{78} Combination therapy with IFN-\(\alpha\) and ribavirin, however, increases sustained virological and biochemical response rates to between 40\% and 50\%.\textsuperscript{79,80} Patients with higher serum HCV RNA levels, severe hepatic fibrosis and significant siderosis, and men, are less likely to respond to treatment.\textsuperscript{76,81}

Hepatitis C virus has become the commonest indication for liver transplantation in Australia and worldwide.\textsuperscript{52,83} Chronic hepatitis redevelops in the majority of transplant recipients, but rarely leads to severe fibrosis or graft failure within the first decade.\textsuperscript{84}

Much remains to be learnt about the molecular virology of hepatitis C

The RNA genome of HCV consists of a single uninterrupted open-reading frame, approximately 9400 nucleotides in length, bracketed by 5' and 3' non-coding regions.\textsuperscript{85,86} Protein translation begins at an internal ribosomal entry site (IRES) in the 5' non-coding region.\textsuperscript{57} The full-length polyprotein of approximately 3000 amino acids undergoes post-translational cleavage by host and viral proteases into HCV core, envelope glycoproteins (E1, E2) and non-structural proteins (NS2, NS3, NS4A/B, NS5A/B).\textsuperscript{88} The core protein is thought to interact with RNA to form the virion nucleocapsid,\textsuperscript{89} while the non-structural regions are likely to have a role in virus replication and encode for proteases (NS2, NS3), a helicase (NS3) and an RNA-dependant RNA polymerase (NS5B).\textsuperscript{90} The virion half-life is between 3 and 5 h, with a clearance and production rate of approximately 10\textsuperscript{12} particles per day, corresponding to 50 particles per infected hepatocyte per day.\textsuperscript{91} Similar to other flaviviridae, HCV is a small spherical virus (diameter approximately 30–50 nm)\textsuperscript{92,93} with a lipophilic envelope.\textsuperscript{94} Hepatitis C virus has been shown to associate with low density lipoproteins (LDL) in human sera.\textsuperscript{95,96} It has been proposed that an interaction between HCV–LDL complexes with LDL receptor is responsible for HCV binding and cell entry.\textsuperscript{97–99} However, the specific mechanism by which HCV particles interact with LDL receptor prior to internalization remains unclear. In addition, HCV E2 protein has been shown to bind the major extracellular loop of human CD81, a cell surface molecule expressed on virtually all nucleated cells.\textsuperscript{100} However, recent evidence suggests that this interaction is not important for HCV entry into cells.\textsuperscript{99,101,102} Hepatitis C virus envelope proteins may be important for fusion with the endosomal membrane through binding alternative, unidentified, cell surface proteins.\textsuperscript{103}

Diagnosis of hepatitis C is based on serology and detection of viral RNA by PCR

Serological assays for diagnosis of HCV were initially based on the detection of circulating antibody reactive to C100-3, a recombinant epitope derived from NS4.\textsuperscript{3} These assays lacked sensitivity and specificity, particularly in samples collected early in the course of HCV infection.\textsuperscript{104} Subsequent second and third generation enzyme-linked immunoblot assays and recombinant immunoblot assays detect circulating antibodies to multiple HCV epitopes, including core, which have been shown to appear earlier in the illness.\textsuperscript{105} Consequently, serological diagnosis has a sensitivity and specificity that approaches 95\% when compared with serum HCV RNA detection by PCR of reverse transcribed cDNA.\textsuperscript{106} There is however, a proportion of HCV-infected individuals who are HCV RNA positive, but negative by serology.\textsuperscript{107} This group
includes a small group of patients who have a delay of over 12 months before seroconversion after primary infection. Despite this, because serum HCV RNA levels fluctuate significantly over time and may give a false-negative result if the rate of viral replication is low or if viral persistence is limited to the liver, PCR testing is generally used to confirm persistent HCV infection only following serological testing for anti-HCV antibodies.

The liver is the principal site of hepatitis C replication

Immunohistochemical studies of liver specimens from patients with persistent HCV infection have demonstrated structural and non-structural antigens located almost exclusively within the cytoplasm of hepatocytes. In situ hybridization to detect complementary negative-strand RNA confirms that hepatocytes are the primary target for intra-hepatic HCV replication. Early reports suggested that only a small proportion of hepatocytes are positive for negative-strand RNA, however, more recent evidence suggests that 25%, or even up to 73%, of these cells may replicate HCV within them. The potential for extra-hepatic replication and the biological significance of negative-strand RNA intermediates in both liver-infiltrating mononuclear cells and PBMC is unresolved. Additional sites of apparent extra-hepatic replication in immunodeficient hosts include lymph nodes, spleen, pancreas, adrenal gland and thyroid.

Hepatitis C has a highly variable genome

The genomic sequences of individual HCV isolates vary by as much as 35%. This nucleotide sequence heterogeneity is unevenly distributed over the genome. The most highly conserved region is at the terminus of the 5′-non-coding region. The proximal 3′-non-coding region varies in nucleotide sequence and length, downstream of which most isolates have a poly(U) stretch followed by a highly conserved 98-nucleotide tail. The conserved non-coding sequences are believed to be important for regulation of viral replication and gene expression. The putative core is the most highly conserved HCV protein, followed by conserved sequences in NS3 and NS5. The envelope proteins contain conserved elements, but overall these proteins are highly variable. A domain of approximately 28 amino acids at the amino-terminal end of E2 is so diverse among different HCV isolates that it has been labelled the hypervariable region (HVR1). However, despite significant variation at the nucleotide level, it retains a conformational structure that suggests it is involved in interactions with negatively charged compounds such as glycosaminoglycans and phospholipids. This is consistent with the hypothesis that HVR1 is important in interactions between HCV and the cell surface.

Hepatitis C exists as a dynamic quasispecies

Typically, RNA viruses show high mutation frequencies because of an error-prone viral RNA polymerase that lacks proof-reading 3′-5′ exonuclease activity. For HCV, the rate of nucleotide misincorporation has been calculated as approximately 10⁻³ base substitutions per genome site per year. The resulting genetic diversity defines the evolution of a quasispecies: a complex population of closely related HCV variants circulating simultaneously in each individual patient. The quasispecies encompasses a predominant ‘master’ genome, which is believed to have a superior replicative capacity, and a multitude of distinct ‘minor’ genomes. Generally, HCV quasispecies vary by less than 5% in nucleotide sequence. The generation and evolution of the quasispecies is likely to have important implications for escape from immune surveillance, generation of drug resistance and vaccine failure. Considering the potential role of the HVR1 in cell entry, as discussed above, and its high rate of nucleotide diversity, it is conceivable that different components of the quasispecies within a single patient may display different cellular tropism. This may explain the observation that circulating quasispecies do not always reflect those of the intrahepatic virus.

The six hepatitis C genotypes may vary in pathogenicity

Phylogenetic analysis of HCV isolates worldwide has lead to the classification of HCV variants into six distinct genotypes based on the NS5 region. Hepatitis C virus genotypes vary by as much as 30% in nucleotide sequence. Multiple, closely related isolates within these six genetic groups have been identified and are classified into subtypes (for example, 1a, 1b). It is not yet resolved as to whether more recently isolated isolates from South-East Asia should be classified within the established six genotypes or represent three distinct, additional genetic variants.

Detection of viral nucleotide sequence variations can be achieved using polymerase chain reaction (PCR) sequencing, type-specific primers, heteroduplex mobility analysis, selective hybridization and restriction fragment length polymorphism techniques targeting well-conserved areas, typically the 5′-non-coding region, of the HCV genome. Alternatively, serological methods that rely on differential detection of antibodies to antigenic sites, for example NS4, can be used to type HCV isolates. Genotype-specific changes in one region of the genome predict changes in other areas, thus, detection of type-specific antibody to NS4 peptides has been shown to correspond with PCR-based genotyping in almost all cases. The prevalent HCV genotypes vary in different geographical regions. Genotype 1 is widely distributed; however, in the United States genotype 1a predominates, while in Japan type 1b and 2a are most common. Genotype 4 is essentially restricted to the Middle East and central Africa, genotype 5 to South Africa and genotype 6 to Hong Kong. The distribution of given genotypes can vary in a single area over time. Over the past 30 years in Australia there has been an emergence of genotype 3, which is endemic in South-East Asia and India, and a corresponding relative reduction in the prevalence of genotype 1.

All currently recognized HCV genotypes are hepatotropic and pathogenic. However, it has been suggested that different genotypes do vary in their infectivity and pathogenicity, thereby influencing the rate of progression to cirrhosis and the risk of hepatocellular carcinoma. Specifically, genotype 1b has been reported to be associated with higher
Hepatitis C persists despite a vigorous immune response

In contrast to other flavivirus infections and HBV, the majority of adults with primary HCV infection develop persistent infection. In addition, HCV can be responsible for multiple episodes of acute disease in an individual. Repetitive exposure of chimpanzees to heterologous and homologous HCV strains results in the reappearance of viraemia, as well as histopathological evidence of acute hepatitis attributable to reinfection rather than reactivation of the original strain.41 Similar observations have been made in multiply transfused thalassaemic children.170 As a DNA replicative intermediate has never been demonstrated in the HCV life cycle, there is no evidence to suggest that persistent HCV infection is related to viral integration into the host genome. Persistence must, therefore, relate to the inability of the host to mount an effective immune response, to viral factors that facilitate immune evasion, or to a combination of these factors.

There is no evidence to suggest that individuals with persistent HCV infection are globally immunosuppressed.171,172 There is, in fact, considerable evidence that the majority of HCV-infected patients are capable of mounting polyclonal cellular and humoral immune responses directed at both structural and non-structural viral proteins. The recognized components of the adaptive anti-HCV immune response are summarized in Figure 3 and are discussed below.

Immune responses may mediate liver injury in hepatitis C

In persistent HCV infection the immune response may contribute to hepatocellular injury. Immunosuppression of patients is generally associated with a transient normalization of serum transaminase levels and a surge in viraemia, while removal of immunosuppression can lead to an acute exacerbation of hepatitis.173 In addition, immunohistochemical studies in HCV infection indicate that T lymphocytes predominate both in the expanded portal tracts and inflammatory lobular infiltrates, suggesting that these cells are important mediators of HCV-related liver disease.41

The mechanisms of accumulation of effector cells in the liver in the setting of HCV are largely unexplored. The T-cell chemoattractant, interferon-inducible protein 10 (IP-10) is strongly expressed in HCV-infected liver.174,175 Our recent studies have demonstrated that hepatocytes are the major source of this chemokine, and that levels correlate with the degree of lobular inflammation.176

CD4+ helper T cells are an important component of the immune response to hepatitis C

Antigenic peptides derived from the cleavage of exogenous viral proteins are recognized by CD4+ T-helper cells in association with MHC class II molecules on the surface of APC.177 T-helper lymphocytes have an immunoregulatory function through the secretion of lymphokines that support either cytotoxic T lymphocyte (CTL) generation (the T-helper type 1 cytokine profile: IL-2, IFN-γ) or B-cell function and antibody production (the type 2 profile: IL-4, IL-5, IL-10).178 Studies of the anti-HCV T-helper cell response, as detected
by measuring PBMC proliferation in vitro in response to recombinant HCV proteins, have revealed that between 50% and 75% of individuals with persistent HCV infection have CD4+ T-cell responses to core, NS3, NS4 or NS5 proteins. A hierarchy of T-cell responsiveness to HCV proteins has been defined with core being the most immunogenic followed by NS4. Rather than representing the lack of T-helper lymphocyte activity, the absence of detectable lymphoproliferative responses to HCV in a minority of individuals with persistent HCV infection, may either relate to antigen-specific CD4+ cells present in the circulation at a frequency below the sensitivity of the assay, or lymphocytes that recognize variable regions of HCV proteins. An alternative explanation is compartmentalization of HCV-specific CD4+ T cells to the liver, as demonstrated in one study. Viral persistence in the face of significant T-helper cell proliferation at the site of viral replication may relate to the observation that, despite 40–80% of liver-infiltrating CD4+ cells expressing activation markers, just 1% of these cells were specific for HCV antigens. However, healthy subjects with lower levels of viraemia exhibit more vigorous proliferative responses to HCV antigens than those with progressive disease, particularly against core protein. This suggests that CD4+ T cells are indeed likely to influence HCV replication in patients with persistent HCV, probably by facilitating other effector mechanisms.

The notion that CD4+ T lymphocytes may either directly or indirectly mediate tissue destruction is supported by the observation that a greater parenchymal concentration of activated CD4+ lymphocytes corresponds with more severe hepatitis. However, in one study, in vitro proliferation of T-helper cells was associated with a lower grade of liver inflammation, suggesting that CD4+ cells may be involved in host protection rather than liver injury. Similarly, higher levels of PBMC proliferation in response to HCV antigens in patients after liver transplantation have been shown to predict less severe histological changes due to HCV recurrence.

An association between HLA class II genotype (for example, HLA DRB1*01) and HCV clearance in primary infection, suggests that CD4+ cells help play a critical role in facilitating clearance after HCV exposure. Furthermore, a vigorous CD4+ T-cell proliferative response from PBMC to multiple HCV proteins has been shown to predict disease resolution. Similarly, viral clearance after a course of IFN-α therapy and combination IFN-α and ribavirin treatment appears to relate to the induction of a vigorous T-helper response. Rather than directly achieving viral clearance, it is likely that T-cell help plays a critical role in facilitating other antiviral immune mechanisms (e.g. CTL) as discussed below.

The CD4+ response to HCV antigens has frequently been shown to persist for up to several decades following viral clearance, without apparent re-exposure to HCV. The response is generally directed at highly conserved immunodominant epitopes within the NS3 and core proteins that bind multiple HLA class II alleles. These features of the host response provide an encouraging backdrop for the development of prophylactic HCV vaccines designed to elicit cellular immune responses.

Neutralizing antibodies do not prevent hepatitis C infection

During primary HBV infection, the humoral response to envelope antigens contributes to the clearance of circulating virus particles. Similarly, in acute flavivirus infections, such as dengue and yellow fever, viraemia is typically terminated by the appearance of conformation-dependent neutralizing antibodies specific for determinants located on envelope glycoproteins. Clearance of yellow fever virus-infected cells is achieved by antibody-dependent complement-mediated lysis of cells bearing non-structural proteins. An effective humoral response, induced either by natural infection or by vaccination, is thought to generate long-lived protective immunity against subsequent infection. With regard to dengue, neutralizing antibodies are virus serotype-specific, allowing reinfection with alternative genotypes, such as dengue-2, after previous dengue-1 infection. Antibodies to envelope glycoproteins are also responsible for the phenomenon of antibody-dependent enhancement, in which pre-existing antibodies against one genotype (e.g. dengue-1) facilitate viral entry via Fc receptors in the context of infection with a new genotype (e.g. dengue-2). This leads to fulminant clinical illness in dengue haemorrhagic fever, and exaggerated neurovirulence in yellow fever and Japanese B encephalitis. This phenomenon of antibody-dependent enhancement has not been studied in the context of hepatitis C.

Immunization of chimpanzees with recombinant envelope glycoproteins is associated with the generation of a vigorous humoral response and control of HCV viral replication following a low-dose challenge with the homologous virus (i.e. the strain from which the vaccine was developed), but does not protect against heterologous HCV challenge (i.e. with another HCV isolate). In primary HCV infection, a humoral immune response also typically results in the generation of isolate-specific neutralizing antibodies. These antibodies bind only native and not denatured proteins, suggesting that they recognize conformational epitopes. As well as osonizing HCV for elimination, antibodies may block viral attachment and impair HCV entry into cells. However, data from chimpanzees and humans suggest that generation of anti-E2 antibodies is not associated with viral clearance. In addition, resolution of primary HCV infection in agammaglobulinaemic children occurs in a similar proportion to the general population. The fact is that most patients with anti-HCV antibodies harbour a replicating virus, are infectious, and have evidence of liver injury. This suggests that antibodies are not protective. However, E2-specific antibodies may be involved in antibody-dependent cellular cytotoxicity (ADCC) against infected host cells. Rather than facilitating viral clearance, ADCC may contribute to hepatocellular damage.

Evolution of the hepatitis C quasispecies may facilitate immune evasion

It has been postulated that the effectiveness of neutralizing antibodies in achieving viral clearance is limited because they have a restricted spectrum of activity against a potentially broad and evolving viral quasispecies.
emergence of neutralization-resistant escape variants present in the quasispecies.\textsuperscript{221} Studies of chimpanzees inoculated with RNA transcribed \textit{in vitro} from a cDNA clone have demonstrated that escape mutants need not be present in the infectious dose to establish persistence.\textsuperscript{133} However, in natural infection the selection of pre-existing mutants may occur.\textsuperscript{222} The HVR1 of E2 has been shown to be a critical target for neutralizing antibodies.\textsuperscript{213,223} Emergence of variants \textit{in vivo} with mutations in this region may lead to a loss of antibody recognition of HVR1 epitopes and result in persistent HCV infection.\textsuperscript{224-227} Resolution of primary HCV infection has been associated with stasis of the quasispecies, and, conversely, progression to persistent infection has been associated with sequence changes within the HVR1.\textsuperscript{228} However, other factors are likely to be involved in viral persistence as demonstrated by the observation that HCV can cause persistent infection in chimpanzees in the absence of mutation events in the HVR1.\textsuperscript{229} Evolution of the quasispecies may provide the basis of establishment of persistence by selection for mutations in other parts of the HCV genome, such as alternative envelope and non-structural regions,\textsuperscript{133} which may be the targets for other host response mechanisms as outlined below. Indeed it has been argued that the primary failure in subjects who develop persistent HCV infection is a poor cellular immune response and that quasispecies evolution is the result of persistence rather than the cause. In this model, a lack of T-cell help by CD4\textsuperscript+ cells leads to poor early viral control by CTL, restricted generation of antibody specificities, high selective pressure and immune escape.\textsuperscript{230} Alternatively, the observation that anti-HCV antibodies are not protective may simply relate to the timing of the generation of the humoral immune response in primary infection. Hepatitis C virus clearance has been associated with early antibody generation.\textsuperscript{231} Although there is no evidence in HCV infection, a delay in the production of neutralizing antibodies, and subsequent persistent infection, may relate to the appearance of HCV-specific CTL and the selective destruction of antigen-specific B cells expressing viral peptides in association with MHC class I.\textsuperscript{232,233}

Is the cellular immune response to hepatitis C similar to that seen in hepatitis B?

In hepatitis B, in conjunction with an MHC class II-restricted CD4\textsuperscript+ T-helper response, virally encoded peptides synthesized within infected host cells and presented in association with class I molecules, induce a CD8\textsuperscript+ CTL response.\textsuperscript{177} Polyclonal MHC class I- and II-restricted responses to structural and non-structural proteins are crucial for elimination of HBV-infected cells. \textit{In vitro} studies suggest that the cellular immune response is vigorous and encompasses a broad range of epitopes in acutely infected patients who clear HBV, while the response is more narrowly focused in the 5% of adults who develop persistent infection.\textsuperscript{234-237} In contrast to adults, approximately 90% of neonates develop persistent HBV infection following vertical transmission.\textsuperscript{172,238} Neonatal tolerance to maternally derived circulating HBV e antigen may facilitate persistent HBV infection after perinatal exposure.\textsuperscript{239} However, vertical transmission of HBV also results in milder hepatitis, suggesting that the immune response may contribute to liver damage as well as having a role in host protection.\textsuperscript{15} This is supported by the observation that immunodeficient individuals have a higher rate of persistent HBV, but milder liver disease.\textsuperscript{240} Consistent with this notion of immunologically mediated hepatocyte injury is the observation that both spontaneous clearance and acute exacerbations in patients with chronic HBV hepatitis are associated with stronger CTL activity. The direct hepatocytolytic effect of HBV-specific CTL \textit{in vivo} has been demonstrated by the development of dose-dependant severe necro-inflammatory liver disease following adoptive transfer of HBV-specific CD8\textsuperscript+ T-cell clones into transgenic mice expressing HBV antigens.\textsuperscript{241} Experiments using cytokine-specific neutralizing antibodies have demonstrated that in addition to direct cell lysis, CTL may inhibit intracellular HBV replication and cause liver damage via non-cytolytic mechanisms such as the secretion of IFN-\gamma and TNF-\alpha.\textsuperscript{242} Viral clearance without hepatocyte destruction has been demonstrated in chimpanzees with acute HBV infection.\textsuperscript{243} As the majority of hepatocytes become infected in acute HBV, antiviral cytokines may be important in achieving viral clearance, while avoiding massive hepatocyte necrosis and fulminant hepatic failure, thereby ensuring host survival.\textsuperscript{244} Non-cytopathic clearance of hepatotropic pathogens also occurs in murine cytomegalovirus (CMV) and lymphocytic choriomeningitis virus (LCMV) infection.\textsuperscript{245,246} Cytotoxic T cells specific for non-structural proteins presented in association with MHC class I may also contribute to the termination of flavivirus infections, with Kunjin and West Nile virus.\textsuperscript{247} Flaviviruses can upregulate MHC class I expression on infected host cells, thereby augmenting CTL-mediated cell lysis.\textsuperscript{248}

Different methods used to study cytotoxic T lymphocytes in hepatitis C have produced varied results

Several groups have studied HCV-specific CTL responses in peripheral blood and liver from HCV-infected patients and chimpanzees. Two assay strategies have been employed. The first has concentrated on the peripheral blood CTL response after \textit{in vitro} HCV-peptide stimulation to expand the HCV-specific effector population.\textsuperscript{249-251} HLA-A2-restricted CTL activity in peripheral blood has been studied extensively because of the ease with which peripheral blood T cells are sampled and because of the high frequency of the HLA-A2 haplotype. The HLA-B44-restricted HCV-specific CTL response has been studied in Japanese patients because of an association with persistent HCV infection.\textsuperscript{249} The second strategy has sought to characterize the intrahepatic CTL response after T-cell expansion using non-antigen specific proliferative stimuli, such as anti-CD3 antibodies.\textsuperscript{252,253}

In persistent HBV infection, HBV-specific CTL are generally undetectable in peripheral blood. By contrast, at least one HLA-A2-restricted CTL epitope (of 10 studied) was recognized by expanded PBMC in 97% of patients with persistent HCV infection.\textsuperscript{254} In general, the anti-HCV response was multispecific, with an average of four CTL epitopes being recognized in each patient. These CTL epitopes were located both in the structural and non-structural proteins of the virus.\textsuperscript{171} Given that the major site of HCV replication is believed to be the hepatocyte, and that chronic hepatitis due to HCV is characterized by a CD8\textsuperscript+ T-cell infiltrate, it is not
CTL response was demonstrated in the majority of patients, target cell lysis was assessed in a 51Cr-release assay. Natural killing can be detected in approximately 50% of cases. Intrahepatic and peripheral blood hepatitis C virus (HCV)-specific CTL responses and natural killer activity expanded from a patient with chronic HCV infection. Liver and peripheral blood mononuclear cells were expanded for six days in vitro using autologous cells infected with recombinant vaccinia virus constructs encoding HCV genomic regions (HCV genotype 1a core to NS2 (●) and genotype 1a NS2 to NS5 (□)). Autologous B lymphoblastoid cell lines were infected with the HCV-vaccinia virus constructs and vaccinia virus expressing the Escherichia coli β-galactose gene (vv-Lac, control (■)). Target cell lysis was assessed in a 51Cr-release assay. Natural killer activity was assessed against K562 targets (●). Intrahepatic lymphocytes displayed significant HCV-specific CTL activity against both genomic regions. Significantly less HCV–CTL was detected after antigen-non-specific stimulation reflects the intrahepatic and peripheral blood activity in patients with persistent HCV infection. Liver and peripheral blood mononuclear cells were expanded for six days in vitro using autologous cells infected with recombinant vaccinia viruses encoding HCV genes. A broad and vigorous HCV-specific CTL response was demonstrated in the majority of patients, with substantially higher CTL activity evident with liver-derived mononuclear cells than in peripheral blood (Fig. 4). This suggests that HCV-specific CTL are concentrated at the site of maximal viral replication and tissue injury.

**Cytotoxic T lymphocytes may control viral replication in subjects with persistent hepatitis C infection**

The mechanism by which HCV persists despite the presence of a sustained, multispecific CTL response is not clear. Such a finding is particularly surprising considering that MHC class I, intercellular adhesion molecules and Fas antigen are all upregulated in the liver during HCV infection, and should facilitate cell binding, antigen recognition, and hepatocyte lysis. Although there is no evidence in HCV, one explanation may be that infected hepatocytes are induced to express Fas-ligand and may protect themselves against CTL-mediated injury by destroying virus-specific CTL via the same pathway that CTL use to kill their targets.

Cytotoxic T lymphocytes may play a role in controlling viral replication in patients with persistent HCV infection. Attempts to correlate CTL activity with viral load have produced conflicting results. Our experiments suggest that a vigorous intrahepatic CTL response is associated with lower viral loads. In one other study, the presence of an HCV-specific CTL response in liver-infiltrating lymphocytes was also associated with lower viral load. However, other studies have been unable to confirm this observation. An analysis of peripheral blood responses demonstrated no correlation between HCV-specific CTL precursor frequencies and viral load. However, in a related study the overall combined peripheral blood CTL response against a panel of epitopes was higher in patients whose HCV RNA level was below the threshold of detection, suggesting that HCV-specific CTL may be able to exert some control over viral replication, but not enough to terminate infection. A similar inverse relationship has been demonstrated between viral load and the HLA-B44-restricted peripheral blood CTL response to HCV core antigen.

**Cytotoxic T lymphocytes may contribute to hepatocellular injury in chronic hepatitis C**

The lymphocytic infiltrate characteristic of chronic HCV hepatitis contains a predominance of CD8+ cells over CD4+ cells. While CD4+ cells are confined to the portal and periportal areas, CD8+ cells contribute to the lobular infiltrate. This may suggest that similar to HBV, CTL may contribute to the liver damage in patients with HCV. While some investigators have failed to show any distinct cellular infiltrate at the site of antigen-positive hepatocytes, others have demonstrated CD8+ cells in contact with apoptotic bodies and hepatocytes containing HCV antigens. In addition, studies have demonstrated a correlation between the number of lobular CD8+ cells, serum transaminase levels and the degree of histological inflammation. However, correlations between functional assessments of HCV-specific CTL activity in vitro from persistently infected individuals with liver injury have produced conflicting results. The presence of liver-derived CTL activity was associated with a higher hepatic...
Hepatitis C virus clearance in primary infection may depend on the vigour, breadth and quality of the early host CTL response

Immunity in self-limited HCV infection has been difficult to define as the illness is largely asymptomatic, and thus patients rarely present to medical attention with primary infection. Despite this, the role of cellular immune factors in viral clearance during primary HCV infection has recently been addressed using techniques such as enzyme-linked immunospot (ELISPOT) assessment of IFN-γ production, tetramer and intracellular cytokine staining. In two experimentally infected chimpanzees, a vigorous, multispecific, intrahepatic CTL response, which persisted for over 1 year, and a limited antibody response were associated with viral clearance. These data must be interpreted cautiously given that in contrast to humans, only a minority of chimpanzees develop persistent HCV infection. However, a follow-up study of women who cleared HCV after exposure to contaminated human Rhesus immunoglobulin similarly demonstrated that while circulating HCV-specific antibodies were undetectable in 42% of cases 18–20 years after exposure, memory HCV-specific helper and cytotoxic T-cell responses persisted in 79% and 92%, respectively. Other investigators have confirmed that greater numbers of HCV-specific CD8+ T cells are found in patients with self-limited primary HCV infection, that an early, broadly directed response is critical, and that T-helper and CTL responses persist after resolution of clinical hepatitis. This has not been a universal finding with one report of comparable numbers of HCV peptide-specific IFN-γ producing cells in the peripheral blood of persistently infected patients and recovered subjects. It may be that the responses in the liver differ from activity in blood. In line with this, early homing of HCV-specific CD4+ and CD8+ cells from blood to liver has been shown to be critical in chimpanzees that clear HCV infection. The detection of HCV-specific T cells in anti-HCV negative blood donors, HCV-exposed seronegative healthcare workers, spouses and healthy family members of persistently HCV-infected patients raises the notion that a cellular immune response may remain after the loss of anti-HCV antibodies, or persist in people who never generate a significant antibody response. It is possible that cellular immunity persists due to retained antigen, or even plausibly low-level viral replication in the liver of clinically recovered patients. Alternatively, the response may reflect prior sensitization to non-HCV antigens.

The ability of HCV to evade cellular immunity may relate to the observation that such responses, similar to humoral responses, have a viral-isolate specific spectrum of activity that may be ineffective against divergent strains present in the HCV quasispecies. The emergence of CTL epitope variants early in the course of primary infection may contribute to HCV persistence. Following primary infection in a chimpanzee that subsequently developed chronic hepatitis, liver-infiltrating CTL were identified that recognized an epitope in NS3, however, mutation at a single amino acid within the epitope resulted in the loss of CTL recognition. Mutant epitopes that emerge in this context may function as CTL antagonists and prevent T-cell recognition of the wild-type epitope. However, because the HCV-specific CTL response is generally multispecific, the mere loss of a single epitope should not provide sufficient survival advantage to the mutant. In addition, other flaviviruses have the same error-prone RNA-dependant RNA polymerase, which is responsible for the quasispecies nature of HCV, and yet rarely establish persistent infection. It has been argued that the selection of escape mutants occurs in the setting of pre-existing persistent infection, that is, viral persistence leads to the selection of escape variants, not the converse. The extent to which quasispecies complexity contributes to, or is a consequence of, persistent HCV infection remains to be determined.

Early vaccine strategies provide promise for the control of hepatitis C

It is clear that the quasispecies nature of HCV and the multiple prevalent genotypes pose a major challenge for the development of a protective vaccine. It is generally believed that an effective HCV vaccine would have to induce a vigorous, multispecific cellular immune response in order to eradicate HCV before the selection of escape mutants. Identification of conserved CTL epitopes restricted by multiple HLA alleles may facilitate the design of a vaccine that is immunogenic in the general population. A priming immunization with naked HCV-E2 DNA followed by a booster immunization with recombinant E2 protein (the so-called ‘prime-boost’ regimen) induced CTL activity and protected mice against tumour-expressing E2. Two chimpanzees vaccinated with HCV-E2 DNA also generated strong CTL responses, and after a viral challenge both resolved...
primary HCV infection early. A DNA prime and canarypox-HCV boost strategy using a polycistronic construct from the HCV core to the NS3 region has been shown to broaden the range of structural and non-structural epitopes targeted by HCV-specific CTL and IFN-γ-producing cells in immunized mice.

Anti-viral cytokines regulate the immune response to hepatitis C

The observed pattern of a vigorous CTL response and relatively weaker humoral response predicting disease resolution suggests that HCV persistence also relates to the pattern of cytokine release. In primary HCV infection, viral clearance is more likely in patients displaying a T-helper type 2 cytokine pattern of cytokine release. The levels of IL-2 and IFN-γ are typically found in innate immune responses may play a role in immune evasion by hepatitis C virus proteins, such as core, E2, and NS5. The relatively low requirement for immunosuppression to avoid organ rejection after allogeneic liver transplantation suggests that the liver may be a site for the induction of peripheral tolerance. This predisposition towards immune tolerance may relate to the observation that the liver is exposed to numerous harmless foreign antigens, including food and bacteria, from the intestine. Oral tolerance appears to involve both T-cell inactivation and deletion. The hepatotropic nature of HCV may facilitate the induction of tolerance and viral persistence. Activated HCV-specific T cells may be ‘trapped’ in the liver after antigen recognition on hepatocytes. Cells of the innate immune system may mediate apoptosis of activated cells, thereby terminating their function. Hepatitis C may use liver tolerance to promote its own persistence.

Little is known about the role of innate immunity in hepatitis C

Uninfected human liver contains a high proportion of natural killer (NK) cells, and recently liver has also been shown to contain a significant population of cells with both NK-cell and T-cell phenotypic and functional characteristics, the natural T (NT) cells. These cells feature heavily biased T-cell receptor (TCR) gene usage, CD1d restriction and high levels of cytokine production, particularly IL-4 and IFN-γ. In several murine models of infection, liver NT cells appear to play a key role in the host response. Thus, both NK and NT cells may have significant bearing on the immune response to hepatotropic pathogens such as HCV. Early NK-cell expansion, prior to the proliferation of CD4+ and CD8+ virus-specific cells, has been shown to control viral replication in murine models of viral hepatitis, such as LCMV. The high rate of HCV persistence following primary infection may plausibly relate to the observation that patients who develop persistent HCV infection have impaired NK activity, and few intrahepatic NK cells. Studies of this cell type in the liver of humans or chimpanzees with primary HCV infection are yet to be performed to better inform this debate. Similarly, virtually no data exists examining the potential role of NT cells in initiation or propagation of the host response to HCV.

A number of viral factors may facilitate immune evasion by hepatitis C

Hepatitis C virus proteins, such as core, E2, and NS5, may interfere with the immune response, for example, by inhibition of the interferon-induced protein kinase PKR or binding to the gC1q complement receptor. Persistence may also be based on the kinetics of infection
relative to the induction of immunity. During the early stages of infection a high rate of production of HCV virions may simply exceed the capacity of the immune response. Immunological tolerance may subsequently develop due to ‘exhaustion’ of the response in persistently viraemic patients. Viral persistence may also occur in the face of low levels of HCV replication, small doses of antigen, suboptimal T-cell activation, impaired proliferation and poor IL-2 production. Suppression of viral gene expression by a suboptimal immune response may facilitate persistent infection by reducing viral antigen expression so that infected cells escape immune recognition. Another potential method of immune evasion is replication in an immunologically privileged extra-hepatic site. For example, there is evidence for infection of pluripotent haematopoietic CD34+ stem cells. Lymphoid cells may thereby act as a reservoir of the virus that continually reinfects the liver. As discussed earlier, due to variations in the HVR1 potentially altering the tropism of different components of the HCV quasispecies, and the observation that progression to persistent infection has been associated with evolution of the HVR1 viral persistence may relate to mutations in the HVR1 facilitating HCV sequestration in immunologically privileged sites. In addition, infection of cells of the immune system, for example, dendritic cells, may impair their function. Consistent with this is the observation that dendritic cells recovered from HCV-infected patients display impaired allostimulatory activity.

Hepatitis C virus may be directly cytopathic

In general in flavivirus infection, disease pathogenesis relates primarily to a direct cytocidal effect of the virus on host cells. Severe disease is characterized by high levels of viral replication, rapid accumulation of antigen and an aggressive inflammatory response. The observation that the degree of liver damage in HCV-associated fulminant hepatic failure parallels the level of viral replication supports the notion that HCV may directly contribute to hepatocyte injury. In addition, highly viraemic liver transplant patients on immunosuppression may suffer cholestatic liver disease attributed to a direct cytopathic effect of HCV. The more typical situation in the immunocompetent host with established infection is controversial. Reports in patients with chronic HCV hepatitis have suggested a correlation between more severe histological changes and higher serum and intrahepatic HCV RNA levels, and a greater proportion of hepatocytes carrying negative-strand replicative intermediates. Against the argument for HCV having a direct cytopathic role are other studies that demonstrate no correlation between either intrahepatic RNA levels or the proportion of infected hepatocytes and necro-inflammatory activity. In addition, significant hepatocellular death is not seen in transgenic mice that express HCV core or E2 in the liver, although steatosis (which is also commonly observed in human infection) is apparently induced. It may be that the process of replication, rather than simply the presence of individual HCV proteins, induces HCV-associated cytopathic damage. However, it is well recognized that HCV replication can occur in patients without significant liver damage.

The role of viral complexity as a pathogenic mechanism in hepatocellular injury has also been investigated. The degree of diversity of the HCV quasispecies has been shown to correlate with disease severity, such that the within-patient variation in nucleotide sequence increases with the severity of liver disease. However, this has not been a universal finding and may simply relate to a longer duration of infection. Longitudinal analysis of HCV quasispecies after primary infection has demonstrated that the complexity of the quasispecies may predict the subsequent course of HCV. A decrease in diversity is seen in patients that clear primary infection, and a dramatic increase in patients with rapidly progressive chronic hepatitis. In contrast, greater rates of HCV quasispecies diversification are associated with less severe disease in immunocompromised patients after liver transplantation. The different methods used to describe quasispecies diversity may explain such disparate findings.

Conclusion

Despite a decade of research since the discovery of HCV as an important agent responsible for chronic viral hepatitis, much remains to be defined regarding pathogenesis. Considerable efforts are being directed towards the development of an in vitro system to support viral propagation. Such a system will assist in defining the role of various viral proteins in translation, replication and critical virus–host cell interactions, for example, the means of HCV entry into hepatocytes. With regard to disease pathogenesis, the conclusions to date are largely based on studies of small numbers of chimpanzees, or, because of the difficulties in recruiting patients with primary HCV infection, observations have predominantly been made in subjects followed up long after HCV clearance, or in those who have persistent infection. Thus, key information regarding the evolution of the host response and viral dynamics early after infection is lacking. An additional consideration is the difficulty in sampling human hepatic tissue. Anti-viral immunity in the liver appears likely to differ significantly from the immune response observed in peripheral blood. A prospective and systematic evaluation of immunity, in conjunction with the assessment of viral replication and quasispecies, in a number of patients with primary HCV infection will assist in defining the nature of protective immunity. A better understanding of these immunological correlates of viral clearance will then facilitate immunotherapeutic and vaccine strategies.

Acknowledgements

AJ Freeman is supported by a scholarship from the National Health and Medical Research Council of Australia.

References


175 Shields PL, Morland CM, Salmon M, Qin S, Hubser SG, Adams DH. Chemokine and chemokine receptor interactions provide a mechanism for selective T cell recruitment to specific liver compartments within hepatitis C-infected liver. J. Immunol. 1999; 163: 6236–43.

176 Harvey CE, Post JJ, Kumar RK, Freeman AJ, Lloyd AR, Marinos G. IP-10 is expressed in the liver during chronic hepatitis C virus infection and correlates with lobular inflammation. In: 7th International Meeting on Hepatitis C and Related Viruses.


Anthony DD, Post AB, Lehmann PV, Heeger PS. Comprehensive determinant mapping by elispot assay is capable of fully defining the HCV specific CD8 T cell repertoire in humans with self-limited HCV related disease. *Hepatology* 2000; 32: 303A.


Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection. 

Hepatitis C virus-associated fulminant hepatic failure. 

Serum and liver HCV RNA levels in hepatitis C patients by strand-specific semiquantitative reverse-transcriptase polymerase chain reaction. 

Hepatitis C viral quasispecies and progression of liver disease. 

Antigenic features. 

Relation of disease activity during chronic hepatitis C infection to complexity of hypervariable region 1 quasispecies. 

Hepatitis C viral quasispecies and liver damage in patients with chronic hepatitis C virus infection. 

Hepatitis C viral quasispecies in hepatitis C virus carriers with normal liver enzymes and patients with type C chronic liver disease. 

Relationship of the genomic complexity of hepatitis C virus with liver disease severity and response to interferon in patients with chronic HCV genotype 1b infection [published erratum appears in Hepatology 1999; 29: 1915]. 

Relationship of the genomic complexity of hepatitis C virus quasispecies after liver transplantation: correlation of genetic diversification in the envelope region with asymptomatic or mild disease patterns. 

Hepatitis C viral quasi species heterogeneity in chronic hepatitis C. 

Clinical features. 

Complexity of hepatitis C virus: correlation with clinical and histological features. 


Degree of diversity of hepatitis C virus quasispecies and progression of liver disease. 

Hepatitis C viral quasi species and the hypervariable region 1 of the hepatitis C virus genome. 

Hepatitis C virus-associated fulminant hepatic failure. 

Species specificity of CD34+ hematopoietic progenitor cells in hepatitis C virus chronic carriers. 

Hepatitis C virus RNA levels in serum, liver, and peripheral blood mononuclear cells of chronic hepatitis C patients. 

Levels of hepatitis C virus RNA in serum, liver, and peripheral blood mononuclear cells of chronic hepatitis C patients. 

Species specificity of CD34+ hematopoietic progenitor cells in hepatitis C virus chronic carriers. 

Diversity of hepatitis C virus RNA in the liver of chronic hepatitis C patients: correlation with clinical and histological features. 

Role of hepatitis C virus in the natural history, treatment, and prevention of hepatitis C. 

Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection. 

Hepatitis C virus infection after liver transplantation: clinical and virological features. 

Transcriptional activity during chronic hepatitis C infection to complexity of hypervariable region 1 quasispecies. 

Diversity of hepatitis C virus quasispecies and progression of liver disease. 

Species heterogeneity in chronic hepatitis C. 

Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection.

Species specificity of CD34+ hematopoietic progenitor cells in hepatitis C virus chronic carriers.

Hepatitis C virus infection after liver transplantation: clinical and virological features.

Hepatitis C viral quasispecies and liver damage in patients with chronic hepatitis C virus infection and its significance in interferon treatment. 

Hepatitis C viral quasispecies and liver damage in patients with chronic hepatitis C virus infection. 

Hepatitis C viral quasispecies in hepatitis C virus carriers with normal liver enzymes and patients with type C chronic liver disease. 

Hepatitis C viral quasispecies and liver damage in patients with chronic hepatitis C virus infection. 

Hepatitis C viral quasispecies in hepatitis C virus carriers with normal liver enzymes and patients with type C chronic liver disease. 

Hepatitis C viral quasispecies and liver damage in patients with chronic hepatitis C virus infection.

Species specificity of CD34+ hematopoietic progenitor cells in hepatitis C virus chronic carriers.