New insights into hepatitis C

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ABSTRACT – Hepatitis C infection is characterised by three key features, which are the consequence of a complex interaction between genetic determinants of immune and other host factors and viral characteristics:

1. **A high rate of viral persistence after acute infection resulting from a combination of weak T cell responsiveness and specific viral mechanisms of immune escape.**
2. **Marked interindividual variability in end-organ damage (fibrosis and cirrhosis), probably due to host genetic polymorphisms in genes governing the immune response and fibrosis pathways in addition to viral pathogenicity factors.**
3. **Significant resistance to antiviral therapies. Viral mechanisms of antiviral resistance parallel those of viral persistence, and include the intriguing possibility that hepatitis C may infect immunologically privileged sites such as the central nervous system.**

KEY WORDS: extrahepatic, fibrosis, genetic, hepatitis C, interferon, liver, polymorphism

Chronic hepatitis C (HCV) infection is probably the commonest persistent pathological viral infection in the UK after the herpes and papilloma viruses. Current estimates of the UK prevalence of chronic HCV infection range between 0.4% and 0.8%, suggesting that approximately 250,000 individuals are infected[1]. There is marked variation in the worldwide distribution of HCV, with very high prevalence rates in parts of the Far and Middle East[2].

Acute infection with HCV is rarely symptomatic. However, unlike acute hepatitis A and B, the virus can evade clearance by the acute immune response in most cases, resulting in persistent infection in approximately 80%[3]. This propensity to chronicity is a key factor in HCV infection. The hepatocyte is the primary locus of infection and the liver is the focus of the immune response with a variable degree of lobular lymphocytic infiltration. HCV is not a cytopathic virus, and it is the immune response itself which leads to hepatocyte necrosis and subsequent collagen deposition and fibrosis[4]. Consequently, variability both in the immune response (which may be due to host genetic factors and/or viral factors) and in the fibrogenesis pathways is likely to explain the second key feature of HCV infection: the marked variability in the degree and rate of progression of fibrosis in different individuals over many years.

Chronic HCV infection is often described as asymptomatic, although the impairments in quality of life associated with this infection are equivalent to those experienced by patients with type 2 diabetes mellitus[5]. Symptoms of fatigue, mental clouding, abdominal discomfort and arthralgia are commonplace, and recent studies have suggested a cerebral effect of HCV with evidence of mild cognitive dysfunction[6,7]. The relatively scant attention paid to these symptoms, which are often present in the absence of significant liver fibrosis, is due in no small part to the dominant side-effect profile of current antiviral therapy, namely combination therapy with interferon (IFN)α and ribavirin. The National Institute for Clinical Excellence guidelines currently reserve treatment for patients with significant necroinflammation and fibrosis on liver biopsy[8].

The aim of treatment is to prevent progression of fibrosis to cirrhosis and its sequelae (ie portal hypertension and hepatocellular carcinoma) by sustained viral eradication. Although significant improvements in therapy have occurred, current treatment regimens fail to achieve this in almost half those treated, with overall sustained virological response rates of 50–60% using combination therapy with pegylated (PEG) IFN and ribavirin[9]. The resistance of HCV to antiviral therapy forms the third key feature that this article will address, in terms of the complex interaction between the host immune response and viral factors.

Hepatitis C virus persistence

Persistent infection is the likely outcome after acute infection with HCV, although a minority of individuals mount a successful acute immune response and clear the virus. Dynamic studies of the immune response have been hampered by the asymptomatic nature of the acute infection. A few studies of patients after spontaneous immune clearance of HCV indicate that this response is characterised by vigorous, multispecific CD4+ T cell responses to both structural and non-structural HCV epitopes[10–12]. In contrast, patients with chronic HCV

infection display much weaker CD4+ T cell responses against fewer epitopes. In addition, the peripheral circulating and intrahepatic cytotoxic T cell response in chronic HCV infection is usually suboptimal[13,14]. A strong and persistent cell-mediated response appears to be more important than the humoral immune response in preventing viral persistence, although prolonged high antibody titres were associated with spontaneous viral clearance in one report[15]. In chronic HCV infection, HCV-specific antibodies appear relatively late at a low titre[16], and in convalescent chimpanzees do not prevent reinfection after rechallenge with homologous HCV strains[17].

These patterns of T cell unresponsiveness and weak humoral immunity in chronic infection imply that HCV has evolved specific mechanisms of immune escape which allow viral persistence in susceptible individuals.

Host genetic factors

Host genetic factors are also implicated in the outcome of acute HCV infection. HLA molecules play a pivotal role in antigen presentation to CD4+ T cells, immune modulation and cytotoxic clearance of infected cells. HLA genes are polymorphic, and disease association studies have attempted to link HLA alleles with outcome after HCV infection. Successful viral elimination after acute HCV infection has been associated with the major histocompatibility class (MHC) class II antigens HLA-DRB1*1101 and HLA-DQB1*0301 in several independent studies[18,19]. However, even in patients with HLA-DRB1*1101 there is marked variation in the immune response. One study in HLA-DRB1*1101 individuals showed persistent epitope-specific memory IFN-γ-producing CD4+ T cells in patients who had cleared the virus and an almost complete absence of such cells in those with persistent infection[12]. Furthermore, in the persistently infected patients, there was an overall dominance of interleukin (IL)-10 production by epitope-specific T cells. IL-10 is a cytokine that may favour viral persistence by downregulating antigen-specific immune responses.

Host viral factors

The general pattern of T cell unresponsiveness in persistent HCV infection may be a consequence of impairment of antigen presentation and/or T cell responsiveness, in both cases possibly secondary to specific viral factors. Monocyte-derived dendritic cells from patients with persistent HCV infection display a greatly reduced capacity to stimulate T cell proliferation[20]. This was associated with the presence of specific HCV genomic sequences in the dendritic cell cultures, consistent with the view that cells constitute an extrahepatic reservoir for the virus. The function of dendritic cells isolated from long-term responders to IFN therapy was normal. Thus, active infection of dendritic cells by HCV may interfere with antigen presentation, favouring viral persistence.

T cell activation depends on effective antigen recognition, which in turn requires both binding of antigenic peptides to MHC molecules and the interaction of the T cell receptor (TCR) with the MHC-antigen complex. Genetic mutation of the peptide epitopes may disrupt these interactions, leading to a loss of affinity of the peptide for the MHC molecule or TCR, followed by a loss of immune recognition. This viral strategy is particularly relevant to HCV which, as a result of the limited fidelity of its RNA polymerase, generates a large number of genetically distinct variants or mutants. In any one individual, the pool of circulating HCV variants is known as the quasispecies. In a rare study of the evolution of the quasispecies in acute resolving HCV infection, the quasispecies diversity markedly reduced immediately before viral clearance, whereas an increase in diversity was associated with viral persistence[21].

In addition to generating escape mutants, specific epitope mutations may result in immunological antagonism, whereby MHC and TCR engagement occurs but renders the T cell unresponsive[22]. It has also been suggested that T cell apoptosis may contribute to HCV persistence through a number of possible mechanisms, including an effect of HCV core protein on nuclear factor (NF) κ6B activation[23]. It is therefore likely that a combination of mechanisms lead to T cell hyporesponsiveness and tip the balance in favour of the virus.

Immunologically privileged sites

Recent studies have suggested that the infection of immunologically privileged sites may be an additional strategy employed by HCV to evade immune clearance. Tissues relatively inaccessible to circulating cytokines, and through which there is limited lymphocyte traffic, may provide a reservoir of virus to reinfect the liver, particularly in the context of suboptimal clearance from the liver. Reports of HCV replication within the central nervous system, based upon the demonstration of a distinct brain quasispecies[24] and the detection of HCV-negative strand RNA by a strand-specific assay[25], support this concept. Although the sequestration of virus within the brain may not result in immune-mediated organ damage, the presence of cerebral magnetic resonance spectroscopy abnormalities and cognitive impairment in viraemic patients with HCV infection suggests that there may be some low-level immune activation[26,7]. Other potential immunologically privileged sites where HCV has been detected include the ovary[27], adrenal, pancreas, salivary glands[28] and peripheral blood mononuclear cells[29].

Fibrosis

There is a marked variation both in the natural history of chronic HCV and in the time required for an individual to develop liver fibrosis and cirrhosis. Fibrogenesis can be considered to be a continuous process driven by a persistent liver insult. Individuals with HCV who progress most rapidly have an increased drive to fibrosis resulting from either exogenous factors (eg increased viral pathogenicity or co-ingestion of alcohol) or endogenous factors mediating an increased responsiveness to the liver insult (eg gender, age, immune response and genetic make-up). Large epidemiological studies have defined major risk factors associated with rapid

fibrosis to be male gender, excess alcohol and age over 40 years at acquisition of the virus[30]. However, only a small proportion (18%) of the interindividual variability in the rate of fibrosis is explained by the known demographic and environmental factors. The rest must be explained by other differences between individuals. Several general and specific lines of evidence suggest that host genetic factors play a key role. Host genotype determines outcome in a number of infectious diseases (eg malaria[31], hepatitis B[32,33], HCV[19]). The natural history of HCV varies substantially even when age group, gender and viral variables are controlled, as demonstrated in the cohort of Irish women infected through contaminated anti-D (RHO) immunoglobulin[34]. It therefore seems certain that genetic differences between individuals are responsible for much of this variability.

Host genetic risk factors

Genetic risk factors can be identified using disease association studies comparing allele frequencies of candidate genes in carefully phenotyped groups of patients with HCV. The cellular and molecular mechanisms of the fibrosis pathway are well described[35,36] and a number of key genetic polymorphisms are beginning to surface.

Powell *et al*[37] demonstrated a significant relationship between inheritance of high transforming growth factor-B1 and angiotensinogen-producing genotypes and the presence of severe hepatic fibrosis on liver biopsy. In support of these findings, angiotensin-converting enzyme inhibition attenuated fibrosis development in a rat bile duct ligation model[38]. There are, however, a number of contradictory reports regarding other genetic polymorphisms associated with liver fibrosis, for example haemochromatosis gene mutations and liver fibrosis in HCV[39,40].

These differences have arisen largely as a result of difficulties in assigning an accurate phenotype. For example, a comparison of individuals at different stages of liver fibrosis may merely reflect duration of infection rather than any propensity to fibrosis. Similar methodological problems are evident in a number of studies looking at HLA polymorphisms and progression of HCV-related liver disease, with comparisons between patients with normal and elevated alanine aminotransferase[41], cirrhosis and chronic hepatitis[42], and between asymptomatic patients and those with symptomatic liver disease[43].

The most accurate approach is to consider the rate of fibrosis as the phenotype[30]. The problems inherent in comparing mild and severe fibrosis are avoided, but accurate data on the duration of HCV infection are required. The rate of fibrosis can be considered to be a complex genetic trait. Multiple genes are probably involved, and those found to influence fibrosis progression are likely to have small additive effects, so large numbers will be required to give adequate statistical power. Studies investigating the role of genetic polymorphisms will need to control for potential viral pathogenicity factors.

Although large studies have previously refuted viral genotype as a determinant of the rate of liver fibrosis, evidence is emerging to suggest that HCV genotype 3 is associated with hepatic steatosis, possibly through a direct cytopathic mechanism. Hepatic steatosis is an important determinant of fibrosis, both in the context of genotype 3 infection and also in non-genotype 3 infection, where a relation between body mass index and fibrosis has been demonstrated[44]. Indeed, host genetic factors associated with insulin resistance and hepatic steatosis may well be important in the progression of HCV-related hepatic fibrosis.

Future studies are likely to report positive associations between genetic polymorphisms and rate of fibrosis in HCV infection, possibly even in alleles not currently recognised in models of fibrogenesis. Thus, identification of specific genetic factors may not only give new insights into the molecular mechanisms of fibrogenesis but also assist in prognosis, therapeutic decision-making and even therapeutic discovery.

Drug resistance

The most efficacious antiviral therapy in HCV infection is combination therapy with PEG IFN and ribavirin. The improved pharmacokinetic profile of IFN in the PEG form, together with the addition of daily ribavirin, have resulted in an improvement in overall sustained response rates from approximately 25% to 50%[9]. A significant number of patients continue to be resistant to therapy or respond initially but relapse after the cessation of antiviral therapy. The antiviral mechanism of IFN consists of two main effects:

1 The production of antiviral enzymes such as 2',5'-oligoadenylate synthetase and a double-stranded RNA-dependent protein kinase (PKR).
2 Augmentation of the cell-mediated immune response[45].

To a large degree, the mechanisms of antiviral resistance parallel those outlined earlier for viral persistence and represent a complex interplay between viral and host factors.

Host factors

Age is an important determinant of disease progression, but its importance with regard to IFN response is less clear, although female gender and young age appear to be favourable predictive factors[46]. Race may also be important, with lower response rates in African-Americans than Caucasians, Hispanics and Asians[47,48]. In support of a genetic basis to treatment response there are reports of various HLA associations, but these have not been confirmed in subsequent studies[49]. IL-10 polymorphisms have been associated with sustained response in a single study[50].

Viral factors

Although host genetic factors are likely to play a role in treatment response, there has been more success elucidating the viral factors underlying failure of response to treatment.

The current best predictors of treatment response are viral load and HCV genotype[51,52]. A number of studies have shown that high levels of pretreatment viraemia are associated with a

poorer response to both IFN monotherapy and combination therapy. Furthermore, an early and rapid decline in HCV RNA after the start of treatment predicts a higher rate of sustained response. HCV genotypes 2 and 3 are associated with a better treatment response than genotpye 1, the most recent reports claiming that they show a sustained virological response of up to 80%[9]. The relative resistance of genotype 1 to treatment may be due either to a higher replicative ability with higher viral loads than the other genotypes or to a difference in the host response.

If IFN monotherapy is started during the acute infection, response rates are nearly 100%[53]. Although this approach may not be practical outside the setting of occupational needlestick injury, it provides an important insight into how HCV may evade the effects of treatment in the chronic setting. An IFN-induced antiviral state in hepatocytes, together with an augmented immune response *early* in the course of infection, may allow rapid viral clearance before the virus can mutate to IFN-resistant forms. Many studies have examined mutations in the HCV genome and quasispecies dynamics during IFN therapy. Most quasispecies studies have focused on the most variable region of the envelope 2 glycoprotein, the hypervariable region 1 (HVR 1), which can be recognised by neutralising antibodies and elicits a specific CD4+ T cell response. The baseline quasispecies diversity does not appear to predict response to therapy[54]. However, a reduction in the quasispecies diversity early in therapy, with the emergence of an increasingly homogeneous population, is associated with a sustained viral response. In contrast, the quasispecies diversity does not change significantly in non-responders, although there is emergence of new strains. The early reduction in diversity in sustained responders is likely to reflect a more successful and balanced immune response, but it is unclear whether this occurs because of a reduced viral capacity for immune evasion or enhanced immune recognition.

Interferon sensitivity determining region

HVR 1 interacts with one of the IFN-induced peptides, PKR, and may mediate IFN resistance[55]. However, more attention has been paid to a non-structural (NS) region of the HCV genome, NS5a, which may contain an IFN sensitivity determining region (ISDR). An initial study in Japanese patients suggested that a low degree of genetic diversity in the ISDR (relative to HCV 1b wild type) conferred IFN resistance on the virus, whereas mutations in this region were associated with IFN sensitivity[56]. Furthermore, NS5a interacts *in vitro* with PKR to repress its activity[57]. Thus, it was suggested that the IFN-induced antiviral state could potentially be reversed by inhibition of PKR by specific ISDR sequences. However, the early Japanese studies could not be replicated in most studies from Western countries[58]. In addition, specific ISDR sequences in pretreatment isolates have not been correlated with sustained HCV clearance[59], and experiments with NS5a-expressing cell lines showed contradictory results with regard to the inhibition of antiviral activity by NS5a protein derived from IFN responder and resistant patients[60].

Key Points

Hepatitis C infection is characterised by a high rate of viral persistence after acute infection, marked variability in the rate and degree of liver fibrosis between individuals, and marked resistance to current antiviral therapies

These important features of hepatitis C infection are the result of a complex interaction between host and viral factors

Viral persistence results from a combination of weak T cell responsiveness and specific viral mechanisms of immune escape

Variability in rates of fibrosis and cirrhosis is likely to be due to host genetic polymorphisms in genes governing the immune response and fibrosis pathways in addition to viral pathogenicity factors

Viral mechanisms of antiviral resistance parallel those of viral persistence and include the intriguing possibility that hepatitis C may infect immunologically privileged sites such as the central nervous system

The issue remains controversial. It is likely that other regions of NS5a[61], and indeed of the HCV genome[55], away from the ISDR are important in the interaction between the virus and IFN-induced proteins in mediating antiviral resistance.

Mutations within the HCV 5' non-coding region have an effect on protein translation mediated by the internal ribosomal entry site of the virus. Mutations in this region may confer tropism to particular cell types (eg lymphocytes)[62]. This may serve as an additional mechanism to evade the antiviral effects of IFN if these mutations allow access to an immunologically privileged site such as the brain. Such a mechanism may explain relapse after an apparently successful response to IFN. This mechanism is supported by current quasispecies data, which show that in responder-relapsers there is high baseline quasispecies diversity[54], possibly due to contributions from the liver and extrahepatic sites, and that after relapse, a minor pretreatment variant emerges to be the dominant species.

Conclusion

The accumulating evidence suggests that the important features of HCV infection, namely persistence, end-organ damage and response to therapy, are all determined by a complex interaction between host factors, both immune and non-immune, and viral factors. It is by understanding the nature of these interactions that effective long-term preventive and treatment strategies can be developed.

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