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Review

Immunological determinants of the outcomes from primary hepatitis C infection

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Abstract. Individuals infected with hepatitis C virus (HCV) have two possible outcomes of infection, clearance or persistent infection, determined by a complex set of virus-host interactions. The focus of this review is the host mechanisms that facilitate clearance. Strong evidence points to characteristics of the cellular immune response as the key determinants of outcome, with evidence for the coordinated effects of the timing, magnitude, and breadth, as well as the intra-hepatic localisation of CD4+ and CD8+ T cell responses being critical. The recent discovery of viral evasion strategies targeting innate immunity suggests that interferon-stimulated gene products are also important. A growing body of evidence has implicated polymorphisms in both innate and adaptive immune response genes as determinants of viral clearance in individuals with acute HCV.

Keywords. Hepatitis C, infection outcomes, T cells, cytokines, antibodies, interferon-stimulated genes, genetic polymorphisms.

Introduction

Hepatitis C virus (HCV) is the sole member of the genus *Hepacivirus* in the family *Flaviviridae*. This virus infects 3% of the population globally, resulting in chronic infection in the majority of cases [1, 2]. Humans are the only natural host for the virus, although it can be transmitted experimentally to chimpanzees [3]. The major morbidity and mortality associated with HCV are attributable to chronic hepatitis, which results in progressive fibrosis ultimately resulting in cirrhosis, liver failure and an increased risk of hepatocellular carcinoma [4]. The latter complications result in HCV being the leading indication for liver transplantation in the developed world [5].

Transmission of HCV is parenteral (i.e. blood-to-blood), predominantly associated with re-use and sharing of injecting devices, amongst injecting drug users (IDU) in the Western world, and in sub-standard health care settings in parts of the developing world [1, 2]. The prevalence of infection amongst IDUs increases with duration of risk behaviour, reaching 50–80% in longstanding injectors [6, 7]. Annual incidence rates of up to 40% amongst IDUs have been documented [8, 9]. Other parenteral routes of transmission include blood transfusion (which was prevalent before the introduction of antibody and nucleic acid screening), and tattooing [10]. Approximately

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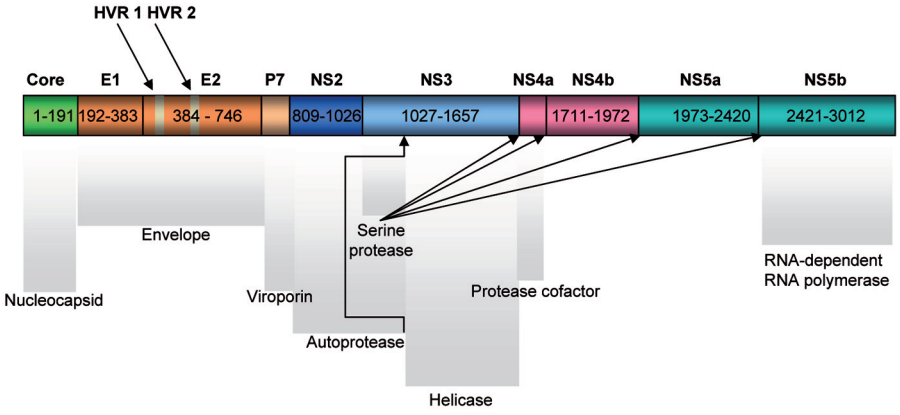

Figure 1. HCV genome, polypeptide structure and protein functions. The HCV genome consists of an open reading frame of approximately 9400 base pairs, which is translated as a single polypeptide of approximately 3000 amino acids. The polypeptide is cleaved to produce 10 mature proteins as indicated, including the four structural proteins: core, E1, E2, and p7; and the six non-structural proteins, NS2, NS3, NS4a, NS4b, N4a, NS5B. The functions of the protein products and the position of the hypervariable regions (HVR) 1 and 2 are shown.

6% of infants born to HCV infected mothers become infected via exposure of the neonate to maternal blood during delivery [11, 12]. Sexual transmission amongst heterosexual couples is extremely rare [13]. However, studies of acute HCV infection occurring in HIV-positive men who have sex with men (MSM) have found many without risk factors for parenteral transmission (reviewed in [10]), suggesting that sexual transmission may occur via mucosal routes in this setting.

Cellular entry of HCV occurs via an initial interaction between the virion and the widely expressed tetraspanin molecule CD81, as well as the scavenger receptor B-1, and then with other intercellular adhesion molecules, the claudins [14]. Hepatocytes are the primary target for HCV infection, although viral genomes and antigens are detectable in sinusoidal endothelial cells and Kupffer cells, as well as leukocytes in the peripheral blood, B lymphocytes in lymph nodes, epithelial cells of the gut, and in the brain [15–18]. In individuals with chronic infection, the viral load in the serum correlates closely with that in the liver, indicating that the liver constitutes the major site of replication [19].

The virus is an enveloped particle consisting of a single positive strand of ribonucleic acid (RNA). The genome encodes a polyprotein of 3010 amino acids which is processed by cellular- and virally-encoded proteases into four structural proteins (core, p7 and the envelope glycoproteins, E1 and E2,) and six non-structural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Fig. 1) [20]. The non-structural proteins are released after cleavage by the viral proteases, NS2–3 and NS3–4A. The structural proteins are cleaved by host endoplasmic reticulum signal peptidases. The genome also contains an overlapping reading frame that may lead to synthesis of an additional protein called the alternative reading frame protein (ARFP). Infected subjects do develop an immune response against this protein, but its role in the virus life cycle and pathogenesis is unknown [20]. HCV has no stable genomic intermediate, and therefore requires continual production of viral RNA and protein in the cellular cytoplasm. The viral RNA polymerase (NS5B) has no proof-reading capacity, leading to a high mutation rate and production of a diverse quasi-species [21–23]. Evolution of the quasi-species has been shown to facilitate emergence of mutants under the selective pressures of host immunity, such as cytotoxic T cell recognition [24, 25], and of antiviral drugs [26]. The most variable region of the genome is the hypervariable region one (HVR1) of the E2 gene. Mutations in this region have been associated with escape from neutralizing antibodies [27, 28]. The half-life of the HCV virion has been estimated at 3–5 hours, with a clearance and production rate of ~10^{12} particles per day [21, 23, 29]. Based on the assumption that 10% of hepatocytes are infected, viral production occurs at the rate of approximately 50 virions per hepatocyte per day [23]. Six main genotypes (1 to 6) and several closely related isolates, termed subtypes (e.g. 1a, 1b), have been recognized, which vary in geographical location and in response to antiviral therapy [30, 31]. Genotypes 1, 2 and 3 are most prevalent in developed nations, whilst in India, South-East Asia and Brazil genotype 3 is most common. Genotypes 2 and 5 are found predominantly in Africa and genotype 6 is also common in South-East Asia. In the Middle East, genotype 4 is the most common, with a particularly high prevalence of HCV infection found in Egypt as a result of a mass vaccination campaign against schistosomiasis where

needles were reused [32]. Recent isolates from South-East Asia have been proposed as new genotypes 7, 8, 9, 10 and 11 [33, 34]. However it has also been suggested that genotype 10 is a divergent subtype of genotype 3, and that genotypes 7, 8, 9 and 11 should be classified as subtypes of genotype 6 [35, 36]. Individuals can be infected with more than one genotype simultaneously or sequentially [37, 38].

Research into viral-host protein interactions has recently progressed rapidly with the development of an effective *in vitro* HCV cell culture (HCVcc) system [39–41], which was previously unavailable [42]. However, studies of the host response to HCV, which appears to be the key determinant of infection outcomes, have been hindered by the fact that the chimpanzee is the only susceptible experimental animal, and is extremely expensive to maintain for laboratory studies. In addition, there are significant differences between the chimpanzee model and the characteristics of the infection in humans [43]. For instance, the hepatic necro-inflammatory activity in chronic infection is more mild in chimpanzees than in humans, and the progression to fibrosis appears slower. Chimpanzees also experience a restricted humoral immune response with a reduced frequency of seroconversion against structural proteins. Nevertheless, valuable analogies with the immunopathogenesis of primary infection in humans have been observed [44].

Infection outcomes

The majority of episodes of primary HCV infection are asymptomatic. The clinical features of symptomatic, acute HCV infection are reported to be similar to those of viral hepatitis of other etiologies. These include nausea, vomiting, loss of appetite and jaundice. In a prospective study of HCV-contaminated anti-D immunoglobulin recipients, only 391 of 888 cases (44 %) with acute infection had been asymptomatic during the acute phase, and those with symptoms reported mild illness only [45]. In cohorts of IDUs, rates of symptomatic hepatitis are very low [46, 47]. One study prospectively identified 19 HCV seroconverters, who made no spontaneous report of symptoms and had no clinical signs of hepatitis [47]. Primary HCV infection is believed to be a very rare cause of fulminant hepatitis, with a case fatality rate of 0.1 % in Italian surveillance data [48].

After initial exposure to HCV, 54–80 % of infected persons develop persistent viremia despite the generation of HCV-specific antibodies detected by enzyme-linked immunosorbent assay (ELISA) and HCV-specific cellular immune responses (Fig. 2) [49–51]. This indicates that the anti-viral immune response is functionally ineffective in the majority of exposed individuals. The ongoing viremia is detected by polymerase chain reaction (PCR) or branched DNA (bDNA) assay.

A correlation between symptomatic disease and viral clearance has been reported, possibly due to a more vigorous immune response, which also results in greater liver injury [50]. In addition, a history of alcohol consumption was associated with a lowered rate of clearance in a study of 77 subjects with acute HCV [49]. A systematic review of studies of the natural history of acute hepatitis C found female gender to be associated with a higher rate of clearance, but no association with viral genotype [51].

The natural history of resolution of biochemical hepatitis in symptomatic primary infection appears to typically span several weeks or longer (Fig. 3). Normalization of alanine aminotransferase (ALT) occurred within three to seven weeks from the time of clinical presentation in one case series [52], and between one and three months after onset of illness in 20 of 24 cases of self-limiting, symptomatic episodes of primary HCV reported in two prospective studies [53, 54].

Clearance does not protect against re-infection, but data in humans suggests that individuals who have previously cleared HCV infection are less likely to develop chronic infection upon re-exposure [55]. A cohort of 164 IDUs who had not previously been infected with HCV (i. e., who were anti-HCV antibody negative) and 98 IDUs who had previously cleared HCV (i. e., who were anti-HCV positive and HCV RNA negative) were followed for a two-year period. New episodes of viremia were detected in 21 % of the subjects without prior infection and 12 % of those who had cleared an earlier HCV infection. Individuals with primary infection had average HCV RNA levels two logs higher than the individuals who became infected after having previously cleared HCV. However, a recent analysis of re-infection episodes amongst a retrospective cohort study of Australian IDUs found no influence on clearance rates [56]. Nevertheless, the data are concordant with the findings in chimpanzees [57, 58]. For instance, one study of four chimpanzees that had cleared an initial infection up to 16 years previously showed that the animals cleared a subsequent infection with either a homologous or heterologous viral strain over an average of 5 weeks post-inoculation, compared to 14 weeks for 2 animals infected for the first time [57]. The level of viremia was two logs lower in the re-challenged animals.

Clearance of HCV RNA from serum does not necessarily reflect viral eradication. Using more sensitive assays than those commercially available,

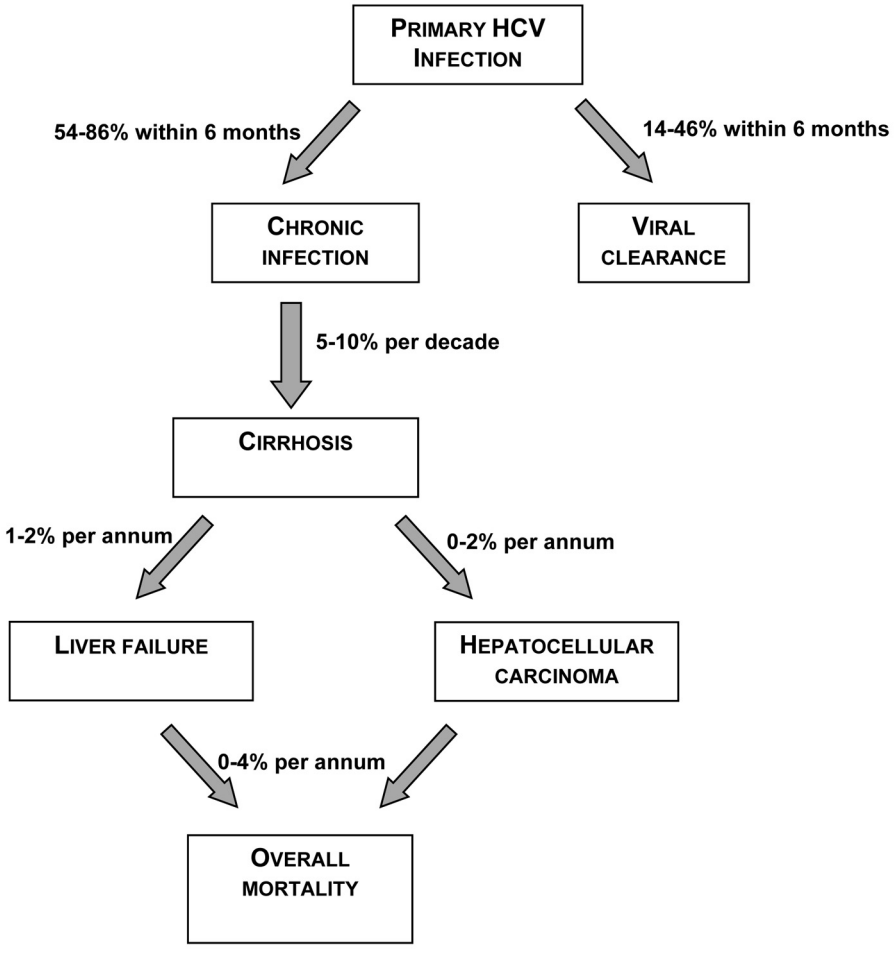

Figure 2. Natural history of HCV disease. Following primary infection, the majority of individuals develop chronic infection which is associated with ongoing necroinflammatory disease in the liver. Chronic HCV infection can lead to progressive fibrosis and ultimately cirrhosis, which may result in liver failure or hepatocellular carcinoma and death.

both positive and negative HCV RNA strands (that is vegetative and replicative strands) have been found in the serum of subjects thought to have recovered from infection, although it is not clear that this finding implies ongoing disease [59]. A study of 100 subjects with abnormal liver function tests of unknown cause (with negative HCV RNA and anti-HCV antibody) found 57 had evidence of HCV RNA in liver biopsy specimens, 48 of which had both positive and negative strands [60]. Eighteen of the 57 also had positive HCV RNA in the peripheral blood mononuclear cells (PBMC) taken at the time of liver biopsy [61]. HCV replication has been detected in B and T lymphocytes, dendritic cells and monocytes from subjects with chronic HCV [62–66], and persistent infection of these cell types after viral clearance has been documented [60, 67–69], although some authors have failed to find comparable evidence [70, 71]. Interestingly, replication in this leukocyte compartment appears to be constrained at low levels by host immune mechanisms [66, 67, 69].

The majority of subjects with chronic HCV have ongoing biochemical and histological evidence of hepatitis, but are typically either asymptomatic or have only mild fatigue [72]. The ongoing necroinflammatory activity in chronic HCV infection drives progressive hepatic fibrosis, with the ultimate development of cirrhosis and consequent liver failure and risk of hepatocellular carcinoma (Fig. 2). The rate of progression in chronic HCV infection varies widely between individuals with estimates of progression to cirrhosis after 20 years of chronic HCV infection ranging from 4–22 % in a systematic review [4]. The most readily available marker of liver injury in both acute and chronic infection is the serum level of the hepatic transaminase enzyme ALT. The ALT levels characteristically fluctuate widely over time in those with chronic HCV infection, correlate poorly with histological disease activity, and may be normal in subjects with cirrhosis [72].

Despite significant improvements in treatment options for chronic HCV over the past decade, the existing standard of care with pegylated interferon-α and ribavirin therapy remains suboptimal, as it achieves a sustained virological response in only approximately one half of those treated [73]. In

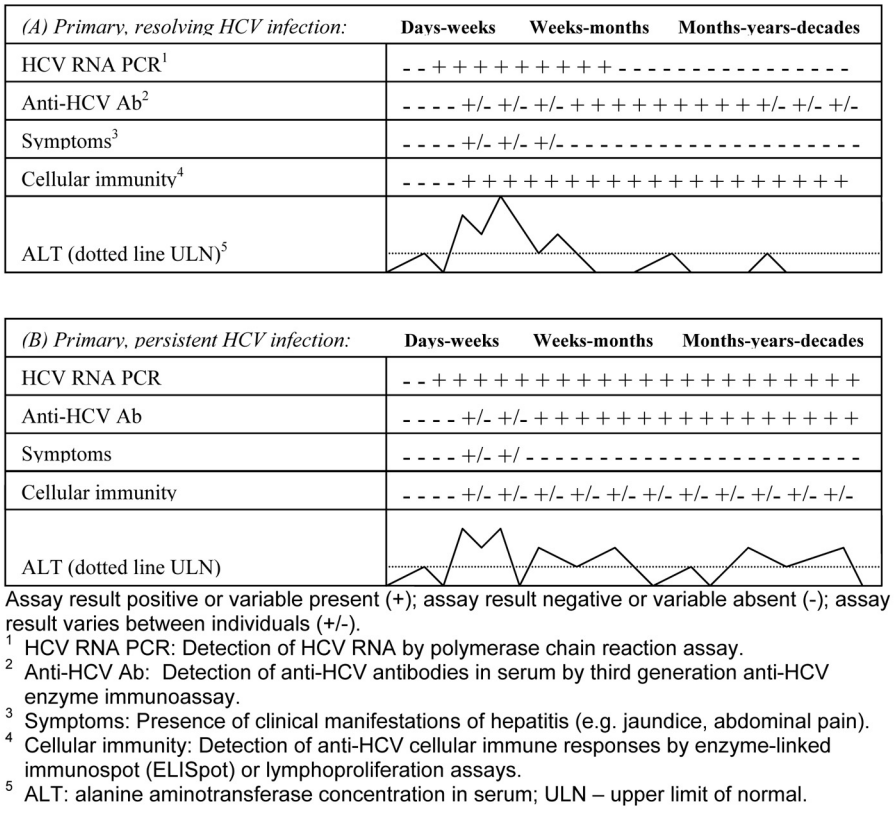

Figure 3. Schematic representations of primary, resolving HCV infection and persistent HCV infection outcomes. *(A)* Primary, resolving HCV infection is more likely to be symptomatic, although it typically has an asymptomatic or minimally symptomatic presentation. Serum HCV RNA is the earliest marker of infection and clears with resolution of the infection (viral clearance). Approximately 50 % of cases are anti-HCV antibody positive at the time of presentation and most cases have persistent antibody detected after viral clearance. Some cases may be seronegative at the time of symptom onset (shown by +/- symbols). Seroreversion may occur in between 7 and 42 % of cases after viral clearance (shown by +/- symbols). Biochemical hepatitis typically develops and the development of jaundice may be more likely to be associated with clearance. The ALT concentration reflecting hepatocellular injury returns to within the normal range with clearance of HCV viremia. Evidence of the development of HCV specific cell mediated immune responses can be detected in the majority of cases and remain detectable for years to decades after clearance. *(B)* Primary, persistent HCV infection is more likely to be asymptomatic. HCV RNA is detected at nearly all time points after onset of viremia. The ALT can return to below the upper limit of normal in some cases, but is typically elevated, at least intermittently. Antibody persistence is usual. Cell-mediated immune responses are detectable, but at a lower frequency than in subjects with primary, resolving HCV infection and may disappear over time.

addition to incomplete efficacy, anti-viral treatment for chronic HCV also requires an extended treatment course over six or twelve months and is associated with significant adverse effects [73]. However, the future prospects for improved treatment options are good with the development of inhibitors of HCV-specific enzymes, NS3 protease and NS5B polymerase, which are likely to be used in combination with existing agents to improve response rates and shorten therapy [74]. In addition, the response rate to antiviral therapy in acute infection (i.e. within the first six months) is considerably higher than in chronic infection (reviewed in [75]).

The remainder of this review focuses on the determinants of the outcomes from primary HCV infection, including consideration of innate and adaptive aspects of the immune response, as well as genetic polymorphisms, which contribute to individual variations in the characteristics of those immune responses. The italicised summary comments highlight the strengths and weaknesses of the existing data, and provide recommendations for further research.

Innate immune responses

Interferon-stimulated genes

Innate immune responses to viral infection generally feature the induction of both cellular responses via natural killer (NK) cells, and antiviral proteins, notably the type 1 interferons (IFN)-α and -β which generate the so-called antiviral state [76, 77], Analysis of the gene expression patterns in the liver of acutely-infected chimpanzees suggests that HCV triggers a

strong type-1 IFN response, featuring expression of many IFN-stimulated genes (ISGs) [78, 79]. However, this ISG response was found to be comparable in animals that cleared the infection and those that become persistently infected. Thus, HCV appears to escape the effector functions of the downstream antiviral target genes that it induces.

Further studies at both the molecular and cellular level are needed to dissect the role of the individual ISGs in the early host response to HCV, and to examine the association between the pattern of induction of ISGs and the outcomes of untreated primary infection in humans.

Natural killer cells

NK cells often play an important role in the innate immune response against viral infection via their potent cytotoxic activity and rapid production of antiviral cytokines, including IFN-γ. Several of the ISGs induced by HCV infection have roles in enhancing the activity of cells of the innate immune system, such as the Mac-2-binding protein (Mac2BP), which regulates activity of NK cells and mononuclear phagocytes [80]. Further evidence of the potential role of innate immune responses in the effective clearance of acute HCV infection is that experimentally infected chimpanzees may clear HCV infection without any demonstrable HCV-specific T cell response [81]. In addition, chronically infected humans have been found to have impaired NK cell function [82, 83]. The precise mechanism for this functional deficit has not been elucidated, and it is unclear whether it is the cause or a consequence of the failure of viral clearance. Interestingly, viral components such as the HCV envelope can inhibit NK cell function [84]. The genetic associations between infection outcomes and alleles of histocompatibility locus antigens (HLA-C) and their receptors on NK cells, the killer cell immunoglobulin-like receptors (KIRs), as outlined below, also suggests that the likelihood of chronic infection may relate to dysfunction of this aspect of the innate response [85].

Further studies are needed to systematically examine NK cell activity in relation to clinical outcomes, including in the liver of acutely infected chimpanzees and in the peripheral blood of human subjects with primary HCV infection.

Viral evasion strategies

A key component of the innate response is triggered when pathogen-associated molecular patterns (PAMPs) on the virus are recognized by specific PAMP-receptors on, or inside the host cell [86]. HCV replication produces double-stranded RNA (dsRNA) intermediates which function as PAMPs. Intracellular and extracellular dsRNA bind with the retinoic-acid inducible gene I (RIG-I) and Toll-like receptor 3 (TLR3) respectively, triggering binding to adaptor proteins and induction of a signalling cascade leading to secretion of type 1 interferons, and thence ISGs [87]. The RIG-I pathway includes the caspase activation and recruitment domain containing (CARDIF) adaptor protein, and transcription factors including interferon-regulatory factors 1 and 3 (IRF-1 and 3). In addition, a second pathway triggered via activation of the Toll-interleukin-1 receptor adaptor protein (TRIF) leads to nuclear factor kappa B (NFκB) activation and thence induction of pro-inflammatory cytokines and chemokines. HCV has mechanisms to evade these innate responses through the actions of the non-structural proteins that appear to favour the establishment of chronic infection. The best characterised of these immune evasion mechanisms involves NS3–4A. NS4A co-factor increases the protease function of NS3 and stabilises it against proteolytic degradation. The NS3–4A protease has also been shown to disrupt signalling from RIG-I by proteolysis of the adaptors, CARDIF and TRIF. This abrogates the induction of the Type 1 IFN and cytokine pathways, thereby favouring viral propagation and presumably establishment of HCV chronic infection [88–90].

The double-stranded-RNA-dependent protein kinase R (PKR) is an ISG which plays an important role in anti-viral defence against RNA viruses such as HCV. PKR autophosphorylates in response to dsRNA and subsequently phosphorylates its substrates, one of which is the α subunit of the eukaryotic translation initiation factor 2 (eIF2-α). This post-translational modification leads to a general inhibition of protein synthesis. In addition, activation of IRF-1 by PKR results in transcription of ISGs. HCV NS5A has been shown to block PKR phosphorylation during RNA replication, which is sufficient to disrupt induction of IRF-1 induced genes. Mutations clustered in or around the PKR-binding domain of NS5A influenced efficiency of replication of culture-adapted HCV RNA replicons [91]. HCV E2 has also been reported to bind to the kinase domain of PKR and inhibit IRF-1 activation [92].

Direct evidence for the activity of these immune evasion pathways in virally-infected cells in vivo is now required, including studies in the liver of acutely infected chimpanzees, and in the peripheral blood of human subjects with acute infection.

Dendritic cells

As well as being the first line in immunological defence against viral infections, the innate response regulates the subsequent adaptive immune response,

primarily via the action of cytokines on the activation of dendritic cells (DCs) and other antigen-presenting cells. Non-productive HCV infection of DCs has been detected in subjects with chronic HCV and impaired antigen-presentation capacity *in vitro* documented in some studies [93–96], but not others [97, 98]. This functional defect may lead to inefficient priming of naïve CD4+ and CD8+ T cells in primary infection, thereby favouring viral persistence [99]. Interestingly, HCV quasi-species sequences cloned from DCs bear an internal ribosome entry site with poor efficiency for translation in cells of liver, lymphoid, or DC origin [65]. It should be noted however that no HCV RNA was detectable in monocyte-derived DCs prepared from chimpanzees with chronic infection, despite a sensitive detection assay [97]. In addition, the frequency of HCV-infected DCs in humans with chronic HCV is very low [99], raising questions therefore as to the significance of the *in vitro* findings on the immunopathogenesis of primary infection *in vivo* [100].

Further examination of the frequency of HCV infection of DCs, including in the liver of acutely infected chimpanzees and in the peripheral blood of human subjects with acute infection, and the functional consequences of DC infection on antigen-specific immune responses are warranted.

Cellular immune responses

Both arms of the cellular immune response, that is CD4+ T helper and CD8+ cytotoxic T cells, have been shown to be important in clearance of HCV infection (reviewed in [75, 101, 102]). The available information from studies in humans with acute HCV infection is generally limited by: the relatively small number of subjects reported and a bias towards those with symptomatic illness; restriction to studies of peripheral blood responses only (rather than intra-hepatic responses); and also by the use of antigens derived mainly from genotype 1 that may not be cross-reactive across genotypes. Chimpanzee studies of acute HCV are limited even further by the small number of animals studied, and by infection with a single viral clone (rather than a diverse quasi-species), but have the advantages of access to intra-hepatic T cells and the opportunity for manipulation of the immune response by depletion experiments. In both subject groups, the majority of the early studies examining acute HCV were cross-sectional rather than longitudinal in design. In general, the evidence indicates that both CD4+ and CD8+ T cell responses appear to contribute to clearance (reviewed in [75, 101, 102]). For instance, in one study of five healthcare workers, the only subject of five to clear acute HCV infection mounted an early, vigorous and sustained CD4+ and CD8+ T cell response [103]. The features of successful immune responses are reviewed below in relation to the specific T cell sub-populations.

CD4+ T cells

Effective antiviral cellular immune responses generally feature induction of CD4+ T cells secreting IL-2 and IFN-γ (so-called Th1 cells), which enhance effector responses of both CD8+ T cells and cells of the innate immune system (i.e. NK cells and macrophages) [104]. Th1 cells directed against HCV are typically identified by enzyme-linked immunospot (ELISpot) assays using recombinant HCV proteins or long synthetic peptides (15–20 amino acids in length) to preferentially stimulate CD4+ (rather than CD8+) T cell responses. CD4+ T cell responses are also commonly measured by proliferation assays. CD8+ T cell responses are similarly measured via IFN-γ ELISpot assay using short peptides (8–11 amino acids). This assay has largely superseded the traditional cytotoxic T lymphocyte (CTL) assay, which measures CD8+ T cell killing of target cells expressing HCV proteins, but has a lower sensitivity [105]. As the precursor frequency of HCV-specific T cells in the peripheral blood is low, some studies have examined responses in cell lines after expansion *ex vivo* using HCV antigens or non-specific stimuli. The relevance of such responses to the context *in vivo* is uncertain. Detection of HCV-specific CD8+ T cells by Class I tetramers allows enumeration of antigen-specific T cells, however concurrent detection of intracellular cytokine stimulation (ICS) is generally not feasible in peripheral blood samples, as the responding cell numbers are too low. The characteristics of a successful anti-HCV T cell response including high magnitude, wide antigenic breadth, as well as early and sustained timing are discussed below. However, chronic infection may still develop despite these features, implying that complete understanding of the characteristics of the ideal anti-HCV cellular immune response remains unresolved.

The breadth of the CD4+ T cell response has generally been shown to be correlated with clearance [54, 106–113]. In a prospective study of 34 subjects with acute infection, five of the eight subjects (63%) who subsequently cleared the virus, had a CD4+ T cell response directed against at least three of the seven peptide pools tested by IFN-γ ELISpot assay, whereas only eight of the remaining 22 (36%) subjects who developed chronic infection responded in the same way [107]. Importantly, one subject who went on to clear viremia had no detectable CD4+ T cell IFN-γ response, implying that this response may not be

essential for clearance. Similarly, a cross-sectional study of 22 subjects with resolved infection, using 301 overlapping peptides spanning the entire HCV polyprotein, found that 89 of the 301 peptides used were targeted by at least one subject (with a range of 3–28 peptides) [110]. In comparison, only seven of 23 (30%) subjects with chronic infection showed one or more positive HCV-specific CD4+ T cell responses, with a mean of one peptide targeted (range 0–8) [110]. Similarly, a study of six subjects with resolved infection found that an average of 10 peptides of the same panel were targeted compared to just one peptide targeted by one of eight subjects with chronic infection [109]. Another cross-sectional study examining proliferative responses to core, NS3, NS4, and NS5 proteins showed that the subjects who had cleared the virus mounted a strong and multi-specific response to all of the proteins, whereas subjects with chronic infection responded weakly or not at all [114]. The magnitude of the CD4+ T cell response has also been associated with clearance [108, 111, 112, 114]. A prospective study of 31 acutely infected subjects using 33 peptide pools showed that subjects displaying more than 390 spot forming units per 2.5×10^5 CD4+ T cells in an ELISpot assay had an eight-fold increase in the likelihood of clearance compared to subjects who did not reach this threshold [112]. Another prospective study found that the number of CD4+ T cells producing Th1-cytokines in response to NS3 and NS4 proteins was higher in the three subjects who cleared infection rapidly than in the seven who developed chronic infection, or the six with transient clearance, only one of whom eventually cleared infection [115].

These findings are generally supported by data in chimpanzees [116]. However, these studies also suggest that the relevance of findings in human studies which focused only on the peripheral blood must be questioned. For instance, a study of five acutely infected chimpanzees showed that there was no difference in the CD4+ T cell proliferative responses in the peripheral blood between the animals that cleared the virus and those that did not, whereas there was a correlation between the intra-hepatic CD4+ T cell responses and clearance [117]. CD4+ T cell depletion before re-infection of two immune chimpanzees resulted in persistent, low-level viremia despite functional intra-hepatic memory CD8+ T cell responses [118]. This finding argues for a cooperative role for both CD4+ and CD8+ T cell responses in facilitating clearance [119].

The non-structural proteins appear to be preferentially targeted by the CD4+ T cell responses in those who clear infection [109, 110, 112–115, 120, 121]. A cross-sectional study of proliferative responses in 29 subjects with chronic infection and 15 with resolved infection showed that the proportion of individuals responding to NS4 and NS5 (as well as core) antigens, was higher among the latter group [121]. Gerlach et al. studied 30 subjects with chronic infection and 38 with resolved infection, and showed a response to at least one of the four proteins tested in those who cleared infection, with NS3 and NS4 being the most immunogenic [120]. In addition, only three of those with chronic infection had a proliferative response to the core protein, compared to all 38 subjects with acute infection [120]. Another cross-sectional study of proliferative responses in 22 subjects with resolved infection and 23 with chronic infection showed that at least three of the six non-structural proteins were targeted by all subjects who had cleared HCV infection, with less frequent responses against the core protein and the variable regions of the envelope protein [110]. In all cases, one or more epitopes on NS3 were targeted, suggesting that epitopes in this protein may be immunodominant [111, 112, 115, 122]. This notion is supported by a study examining IFN-γ ELISpot responses to three peptide pools spanning NS3 (15mers overlapping by 11 amino acids), which found that all 10 subjects who had recovered from infection mounted a strong CD4+ response to all three pools [123]. Similarly, another cross-sectional study using 750 overlapping peptides (15mers) that covered the entire genome in 33 peptide pools found that the 25 subjects with resolved infection displayed on average twice as many CD4+ responses as the 25 subjects with chronic infection. Of these, NS3-specific responses comprised 31% of the cumulative magnitude of the overall response in those with resolved infection, compared with 22% in chronic infection. After normalisation for the relative length of the HCV proteins, NS3 remained the most immunogenic region per amino acid [112].

The kinetics of onset and the durability of the cellular immune response may also be an important determinant of outcome. Both human and chimpanzee studies have demonstrated that many mount a CD4+ response that is initially effective, with a subsequent rebound in viremia and progression to chronic infection [103, 117]. In a study of five healthcare workers with acute infection post-needlestick injury, four developed chronic infection despite a strong initial CD4+ response with control of viremia in two cases [103]. A prospective study of 20 subjects with acute infection found that the number of Th1 cytokine-producing CD4+ cells was higher in the first 12 weeks after disease onset in the subjects with rapid viral clearance, compared to those with only transient or no control of viral replication [115]. The strongest CD4+ T cell response to HCV infection has been

shown to occur within the first six months after infection regardless of outcome [108, 115, 120]. Thus, it appears that a successful CD4+ T cell response, in addition to being strong, multi-specific and targeting the non-structural proteins, needs to develop early and be sustained to achieve viral clearance. This cellular immunity appears to persist for many years after resolution in both chimpanzees and humans [108, 111, 114].

Strong evidence has been provided for a key role for CD4+ T cell responses which are early in onset, high in magnitude, and of broad specificity. Further longitudinal studies are needed to examine the relative contribution of these responses to clearance, including in combination with other aspects of the host response.

CD8+ T cells

Cytotoxic T cells facilitate clearance of hepatitis B virus both by direct cytolysis of infected hepatocytes, and clearance of virus from infected hepatocytes via secretion of antiviral cytokines such as IFN-γ and TNF-α [124]. CD8+ T cells clearly contribute to the cellular immune response against HCV via cytolysis of HCV-infected hepatocytes. For instance, in the study of five healthcare workers with acute HCV infection post-needlestick injury, the first CD8+ T cells to appear in the blood of the subject who cleared infection were CD38+, reflecting an activated status. The appearance of these cells coincided with the appearance of liver disease, but the cells did not produce IFN-γ. Later in the disease course, disappearance of CD38 positivity coincided with detection of IFN-γ production, viral clearance and resolution of hepatitis [103]. Both *in vitro* and *in vivo* evidence indicates that ISG induction by IFN-γ inhibits HCV replication [125, 126]. The role played by CD8+ T cell-derived cytokines in non-cytolytic HCV eradication remains unclear.

Similar to CD4+ T cell responses against HCV, it has been suggested that breadth of the CD8+ T cell response is associated with clearance in both humans and chimpanzees [117, 127–130]. A cross-sectional study of cytotoxic T cell responses using 13 peptides from core, E2, and non-structural protein epitopes found that five of the seven (71%) subjects with resolved infection had responses directed against one or more peptides [130, 131]. In comparison, only four of the 14 (29%) subjects with chronic infection had responses to one or more peptides. Similarly, an early prospective study of CD8+ T cell responses in a subject with acute symptomatic HCV infection using a recombinant HCV-vaccinia based IFN-γ ELISpot assay revealed responses to eight epitopes [127]. More recently, a longitudinal study of 19 acutely infected IDUs was reported, of whom 15 developed chronic infection and four cleared the virus [128]. IFN-γ ELISpot assays using overlapping peptides revealed that the 15 subjects who developed chronic infection had CD8+ T cell responses to a median of 4 epitopes (range 0–10), compared to the four who cleared the virus who had responses to a median of five epitopes (range 4–8). One subject demonstrated responses to seven peptides at day 138 with transient control of viremia. However, the response became progressively narrower, down to four peptides, and chronic infection resulted [128]. This finding is supported by other studies that document that the strongest CD8+ T cell responses occur early in acute infection, with a subsequent decline in both the magnitude and breadth of the response [128, 132, 133]. Generally consistent results were found in a prospective study in five chimpanzees which showed that the animal that cleared the virus mounted an early and multi-specific peripheral CD8+ T cell response, as determined by IFN-γ production in response to seven of the 68 peptides tested, with responses seen in core, E2, and non-structural proteins [117]. This response persisted for at least 68 weeks. However, an animal which transiently cleared the virus also showed a persistent response to eight of the peptides. A much less vigorous response was detected in the other three animals, one of which was also transiently aviremic.

The frequency of CD8+ T cells has also been associated with clearance [127]. A cross-sectional study of seven subjects with resolved infection and 14 with chronic infection found no difference in the number of HCV-specific IFN-γ producing CD8+ T cells as determined by ELISpot [131]. However, other studies have found that HCV-specific CD8+ T cells are more common in patients with chronic infection than in those who have recovered from infection [128, 134], raising the suggestion that ongoing antigenic stimulation is necessary to maintain CD8+ T cells [66, 114]. A cross-sectional study of 20 subjects with chronic infection and 12 who had previously cleared infection revealed that although HCV tetramer positive cells were detected more frequently in unstimulated PBMC from those with chronic infection, they were detected more frequently in stimulated cell cultures from the recovered subjects, implying a weaker proliferative capacity in those with persistence [134].

The kinetics of development of the CD8+ T cell response also appears to be important. For example in a chimpanzee study, the animals that cleared the infection had an earlier intra-hepatic IFN-γ response than those that developed persistent infection [135]. In primary herpes simplex virus infection, activated antigen-specific CD8+ T cells can be detected as early

as six hours after infection [136], in HCV however this takes 7–12 weeks [103, 137]. This delay in the immune response may reflect the fact that HCV infection is primarily localised to the liver, thus HCV antigens may not be available to professional antigen-presenting cells until the infection is relatively well established [136]. Thereafter, the rapid evolution of the viral quasi-species may outpace the generation of the CD4+ and CD8+ T cell responses. Longitudinal analysis of a known immunodominant epitope on NS3 in samples from a subject treated unsuccessfully during acute infection showed evolution of the sequence, with none of the wild type sequence detectable by week 60 [138]. In another subject, development of mutations in this epitope before treatment coincided with a decline in the CD8+ T cell response. Analysis of viral sequences from two subjects acutely infected from a single source revealed that the recipient who developed chronic infection had an escape mutation in an immunodominant epitope [139]. Another study of eight acutely infected individuals defined escape mutations in multiple CD8 epitopes in the seven subjects who developed chronic infection, whereas the one subject who cleared HCV had no substitutions within any recognized T cell epitope at 6 or 12 months after the initial viremia [140]. It has also been hypothesised that rapid viral evolution produces sequences similar to the initial epitope, thus further stimulating immune cells that recognize the old sequence rather than priming new responses [128].

Strong evidence is available for a key role for IFN-γ-producing CD8+ T cell responses which are high in magnitude and broad in specificity, in promoting clearance of viremia in primary HCV. Further studies are needed to relate these CD8 responses to other components of the host response, notably CD4+ T cell responses, and also to define additional CD8+-derived cytokines and their role in non-cytolytic mechanisms of viral clearance.

Regulatory T cells

CD4+CD25+ T cells comprise 2–5% of CD4+ T cells in the peripheral blood and contain a sub-population with the capacity to suppress the proliferation of both CD4+ and CD8+ cells, termed regulatory T cells (Treg) [141]. There are two main subpopulations of Treg: those that occur naturally and those that are induced by infection or other immunological challenge [142, 143]. The Treg sub-population is also marked by the intracellular presence of the forkhead transcription factor 3 (foxp3), which is responsible for the development and suppressive function of Treg [144], and by low level expression of the interleukin-7 receptor, CD127 [145]. Treg have been implicated in various pathological states, including autoimmune diseases [141], and may play a role in development of chronic HCV infection [146], with higher numbers being detected in subjects with chronic infection compared to those who have cleared previous infection [143, 147]. For instance, one of these cross-sectional studies found a higher proportion of CD4+CD25+ cells in peripheral blood of 30 subjects with chronic infection, compared to 15 subjects who had previously cleared infection; these latter subjects also had a lower proportion of Treg than healthy control subjects [147].

The functional effects of Treg on HCV-specific immune responses were investigated in an *in vitro* study which showed that the addition of autologous CD4+CD25+ Treg cells to CD4-depleted PBMC of chronically infected subjects impaired the expansion of HCV-specific CD8+ T cells after seven days of peptide stimulation [143]. The same study showed that tetramer staining of CD4-depleted PBMC after culture with decreasing numbers of CD4+CD25+ Treg cells resulted in suppressive activity that was dose-dependent [143]. Similarly, depletion of CD4+CD25+ cells *in vitro* increased the HCV-specific IFN-γ ELISpot activity in samples from subjects with chronic infection [147].

However, evidence against a role for Treg in promoting the development of chronic HCV infection was recently reported in a prospective study of 27 acutely infected subjects. This study found that there was no significant difference in the proportion of $CD4+CD25^{Hi}$ T cells in the peripheral blood at baseline between the 15 subjects who developed chronic infection and the 12 that subsequently cleared the infection [148]. The frequency for both groups was higher than in healthy controls and did not vary over time. It should be noted however that definition of the Treg phenotype in this study did not include the Foxp3+ or $CD127^{Lo}$ markers. The functional activity of the Treg population was also shown to be comparable early in infection.

A study of two HCV-naïve, eight recovered and six chronically infected chimpanzees showed CD4+CD25+foxp3+ Treg were present in both recovered and chronically infected animals [149]. In addition, HCV-specific IFN-γ ELISpot responses to overlapping peptides before and after CD25+ depletion were compared. The response after depletion was particularly increased in the animals that had recovered from multiple sequential infections compared to those that had recovered from a single infection or were persistently infected.

Definitive resolution of the potential role of Treg in influencing the outcome of primary HCV infection will require further studies utilising the more specific

phenotypic markers, including in the liver of acutely infected chimpanzees, and in the peripheral blood of human subjects with acute infection.

Cells expressing the programmed death (PD)-1 receptor

The PD-1 receptor molecule and its ligands (PD-L1 and PD-L2) function as a co-stimulatory pathway to inhibit T cell activation [150]. Following initial studies in the lymphocytic choriomeningitis virus (LCMV) model of chronic viral infection in the mouse indicating that expression of PD-1 was associated with an 'exhausted' (i.e. non-functional) CD4+ T cell phenotype [151], the pathway was implicated in similar impairment in HIV-infected subjects and shown to be associated with loss of CD8+ T cell responses, higher viral loads, and disease progression [152]. Blockade of the pathway *in vitro* was shown to improve T cell responses [151, 152].

In relation to HCV infection, a cross-sectional study found PD-1 expression to be markedly increased on HCV-specific CD8+ T cells in the peripheral blood of 31 subjects with chronic infection, compared to 11 who had previously cleared infection [153]. This finding was confirmed on both CD4+ and CD8+ T cells in another cross-sectional study [150]. Upregulation of PD-1 has been shown to be induced by HCV core protein [154]. Blockade of the PD1/PD-L1 pathway *in vitro* in samples from subjects with chronic HCV infection, resulted in enhanced CD8+ T cell proliferation and cytokine (IFN-γ and IL-2) production, although the level of enhancement varied between subjects [153, 155]. Comparison of PD-1 expression on T cells extracted from the liver and peripheral blood in 15 subjects with chronic infection showed that 27% of CD8+ T cells in the peripheral blood were PD-1 positive compared to 57% in the liver [153]. These findings are likely to reflect the high intrahepatic antigen load.

Despite these data, evidence against a critical role for PD-1 expression in determining the outcome of primary HCV infection has been provided by a prospective study of 10 subjects [156]. PD-1 was found to be expressed at a high level on HCV-specific CD8+ T cells during the acute phase of infection, regardless of outcome. There was a decrease in PD-1 expression by HCV-specific CD8+ T cells in those who cleared the infection, whereas the level of expression remained high in those who developed chronic HCV.

Clear evidence is available for the role of the PD-1 and PD-L1 pathway in causing T cell exhaustion and impaired antiviral immunity. However, the data in HCV are ambiguous. Further studies in primary infection, including in the liver of acutely infected chimpanzees, and in the peripheral blood of human subjects, are warranted.

Interactions between CD4+ and CD8+ T cells

In chronic viral infections, helper CD4+ T cells are important for the control of viremia through the maintenance of the effector functions of cytotoxic CD8+ T cells. This is mediated both by activation of co-stimulatory pathways and via the production of cytokines, notably IL-2 and IFN-γ [157, 158]. Measurement of cytokine levels in the sera of subjects with chronic HCV has provided inconsistent results, with some studies finding increased levels of IFN-γ and IL-2 [159], and others a prominent increase in IL-4 and IL-10 [160], compared to healthy control subjects. Cytokine production from PBMC stimulated with HCV proteins demonstrated significantly higher levels of IFN-γ and IL-2 in those that had previously cleared infection, while patients with chronic HCV had increased IL-4 and IL-10 production [161].

Evidence for interference in the CD4+ T cell priming environment in acute HCV infection, resulting in reduced effectiveness of the integrated CD4+ and CD8+ adaptive immune response, has been provided by examination of purified HCV-specific, central memory (CCR7+) CD8+ T cells from subjects with acute HCV [162]. These cells displayed poor effector functions *ex vivo*, but proliferated efficiently and differentiated *in vitro* via the supplementation of the cultures with IL-2. In a recent study of 17 patients with acute HCV leading to persistence and 14 with primary infections resulting in clearance [113] this notion was corroborated and extended to a preliminary definition of what constitutes an integrated pattern of CD4+ and CD8+ responses to predict clearance. The study suggested that quantitative CD4+ and CD8+ T cell response thresholds exist, which in combination predict clearance.

Further longitudinal studies in primary HCV are needed to define the cooperative effects of CD4+ and CD8+ T cells in facilitating clearance.

Humoral immune responses

Antibodies typically play a key role in defending the host against pathogens within the extracellular space, with effector mechanisms for viral clearance including neutralization, complement activation, opsonisation, and antibody-dependent cell-mediated cytotoxicity (ADCC). The role of the humoral immune response in the clearance of HCV infection is incompletely defined, although a number of lines of evidence suggest that antibody responses alone are insufficient to control infection in the majority of cases, and also

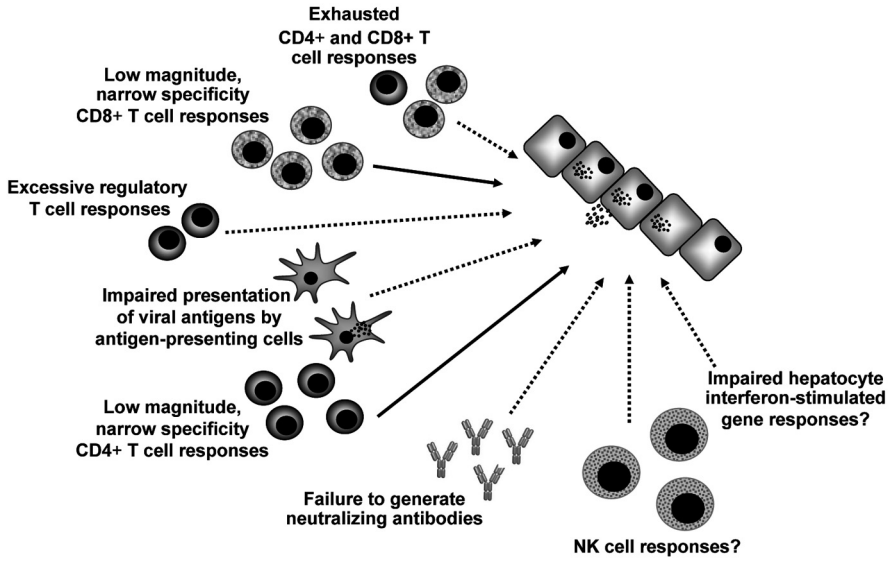

Figure 4. Immunological factors contributing to the development of chronic infection in primary HCV. Dashed arrows indicate possible, but unproven factors. Solid arrows indicate factors with consistent supporting evidence.

that clearance is not dependent on antibody responses in the majority of cases [102].

Several studies have demonstrated that there is restriction of the humoral immune response in HCV infection [163, 164]. These studies have demonstrated that the antibody response generated in HCV infection is of low titer and, with exception of responses against the core protein, the generation of the response is delayed. Another characteristic aspect of the humoral response against HCV infection is the restriction of antibodies to the IgG1 subclass without the usual switching to IgG3 (or IgG4) subclasses that typically occurs with maturation of an antiviral humoral response [163]. Interestingly, there may be an association between the development of IgG2 antibodies and viral clearance. IgG2 responses against core and NS3 antigens were more frequent in subjects with viral clearance in a small study of four subjects who cleared infection in comparison to 23 subjects with persistent infection [165]. Similarly, the ratio of IgG2 to IgG1 antibodies against core and NS5 was greater than 1.0 in those who cleared infection, whereas it was less than one for all antigens in those with chronic infection. This IgG2 predominance has been linked to a Th1 bias in CD4 T cell responses and may be associated with viral clearance [166].

Evidence for the notion that rapid development of an effective humoral response before the emergence of potential viral escape mutations may contribute to viral clearance has been provided by detection of a higher anti-HCV antibody titer in the first two months of infection in chimpanzees who go on to clear infection [116]. Similarly, anti-E2/NS1 antibodies have been detected more frequently in the month after onset of hepatitis in people with transfusion acquired acute HCV infection with subsequent viral clearance than in those with subsequent viral persistence [167]. The early generation of an anti-HVR-1 antibody response was also associated with viral clearance in a small study of subjects with chronic renal failure on hemodialysis who developed acute HCV infection [168]. In another study, the early appearance of anti-HVR-1 antibodies was associated with viral clearance, although the subjects that developed chronic infection also produced antibodies with this specificity [169].

Neutralizing antibodies are generally an important mechanism for control of initial viremia and protection against re-infection in viral infections [170], including hepatitis B virus [124] and flaviviruses [171]. Until very recently, assay systems for detection of neutralizing antibodies against HCV have been limited by the lack of understanding of the cellular receptors for HCV and the nature of the virus-host protein interactions at entry, as well as by the recognized diversity of the HCV envelope sequences. The best available assay systems utilize HCV envelope sequences incorporated within virus-like particles or pseudo-typed viruses which seek to maintain native configurations of the HCV envelope glycoproteins – only a limited number of studies have applied such assays. Nevertheless, reasonable evidence for the presence of antibodies with likely neutralizing capacity have been documented in both humans and chimpanzees infected with HCV, although the absence of reactivity across the quasi-species, and delayed timing of their production in acute infection, imply that these responses are likely to be associated with a lack of efficacy in virological control *in vivo* [75, 172].

Antibody mediated neutralization of infectivity has been demonstrated *in vitro* for homologous strains in chimpanzees [173]. Neutralizing antibodies have been demonstrated to target the HVR-1 of the E2 protein of HCV, and mutations in this region of the virus have been associated with the evolution of the quasi-species leading to escape from neutralization [27, 28]. This area of the genome undergoes a high rate of mutation in acute HCV infection [174]. These and other data imply that neutralizing antibodies provide significant selective pressure on the viral quasi-species, and hence argue for the potential importance of this aspect of the humoral response in viral clearance [175]. Further evidence of selection pressure by humoral responses is provided by the reduced rate of viral mutation in hypogammaglobulinaemic individuals [176]. In addition, the peak of the HCV viral load was inversely correlated with anti-E2 antibodies in a chimpanzee vaccination model [177]. However, some animals cleared HCV infection without developing significant titers of such antibodies, suggesting that they are not necessary for viral clearance. The titer of anti-E2 antibodies was lower in women who had cleared HCV infection after infection with contaminated anti-D immunoglobulin than in those who had persistent viremia [178]. Unfortunately, this was not assessed during the early phase of infection; hence it was not possible to ascertain if the reduced level of anti-E2 antibodies was merely a consequence of a lack of ongoing viral replication or if it represented an early biased pattern of humoral immune response.

Studies have been developed using virus-like particles (VLP) based on retroviral [179, 180] or vesicular stomatitis virus [181, 182] backbones, which bear native HCV envelope glycoproteins and allow assessment of whether antibody neutralises cell entry. Initial studies using this methodology found that neutralizing antibodies were rare in individuals who resolve infection [183–185], although this was not universally observed [186]. However, a recent study using homologous viral pseudoparticles showed that clearance of infection was associated with the rapid development of neutralizing antibodies [187]. Other cross-sectional studies have also found that seroreactivity against HCV VLPs or neutralized pseudotype particle constructs bearing HCV envelope glycoproteins were not associated with HCV clearance [188–191]. Definitive evidence for the role of neutralizing antibodies in promoting clearance of primary HCV infection awaits application of the improved assay systems to samples of both the circulating virus and the concurrent antibodies in the sera from longitudinal case-control subject series.

Further studies using pseudoparticles based on HCV envelope proteins prepared from viral sequences of the dominant quasi-species identified at multiple time points, and the concurrent antibodies in sera collected from subjects with early primary HCV infection who progress to chronicity or clearance, are required to resolve the role of neutralizing antibodies in the outcome of primary infection.

Infection without seroconversion

Strong evidence to suggest that humoral immune responses are not essential for clearance of HCV viremia in primary infection, has come from the demonstration of clearance without the generation of detectable antibody responses in both chimpanzees and humans [192–194]. The first evidence for this phenotype was the report of sustained viremia and subsequent clearance in association with HCV-specific cellular immune responses, but without seroconversion in high risk Australian prison inmates [194]. This phenotype was confirmed in a subsequent report [195]. Subjects that have undergone viral clearance in such a manner may represent a group with 'seronegative immune' phenotype [196]. Interestingly, a polarized cellular response without seroconversion was also shown when two chimpanzees sequentially exposed to 1, 10 and 100 RNA (+) virions developed detectable but transient viremia in the absence of seroconversion after exposure to 1 and 10 virions. In contrast, both animals developed anti-HCV antibodies after exposure to 100 virions, suggesting that viremia without seroconversion may occur after exposure to very low doses of HCV [197]. Although traditional serological responses were not detected in these reports, it remains plausible that an undetected, epitope-specific humoral response was associated with viral clearance before the typical polyclonal antibody response had developed.

Further studies of subjects with the seronegative-immune phenotype are warranted to determine the characteristics of the apparently efficient cellular immune responses which facilitate viral clearance.

Genetic factors

The outcome of HCV infection is likely to be influenced by genetic factors that govern the host immune response. Racial differences in rates of clearance of HCV have been observed, with white subjects more likely to clear HCV viremia than black subjects in a study of IDUs from North America [46, 198]. A higher prevalence of chronic infection was also found in non-Hispanic blacks than in non-Hispanic whites or Mexican Americans [199]. One

Table 1. HLA associations with viral clearance in primary HCV infection.

Reference	Population studied	Alleles or haploptype	Association	Effect size (Percentage RNA negative vs. RNA positive)	OR, RR or Pc
Alric 1997 [216]	French Caucasoids	DRB1*1101	Viral	40 vs. 10	Pc <0.02
	103 RNA +	DQB1*0301	clearance	84 vs. 31	Pc <0.01
	25 RNA –		Viral clearance		
Cramp 1998 [217]	Caucasoids	DQB1*0301	Viral	53 vs. 18	OR 5.09
	Clinic pts vs. HCW	DQA1*03	clearance	51 vs. 18	OR 4.69
	49 RNA +; 55 RNA –	DRB1*04	Viral	47 vs. 16	OR 4.52
	134 controls	One or more of above 3	clearance	69 vs. 27	NS
			Viral clearance		
			Viral clearance		
Minton 1998 [218]	Caucasoid, 3 % Asian	DRB1*11	Viral	31 vs. 8	RR 0.19
	137 RNA +	DQB1*0301	clearance	51 vs. 24	RR 075
	35 RNA –		Viral clearance		
Mangia 1999 [219]	Italian Caucasoids	DRB1*1104	Viral	18.6 vs. 5	OR 4.51
	149 RNA +	DQB1*0301	clearance	53 vs. 29	OR 4.52
	35 RNA –	DRB1*1104-DQB1*0301	Viral	18.3 vs. 4.8	OR 7.38
			clearance		
			Viral clearance		
Lechmann 1999 [220]	German Caucasoids	DRB1*15011	Viral	44 vs. 5.6	RR 13.6
	9 RNA –		clearance		
	18 RNA +				
Barrett 1999 [221]	Irish female anti-D immunoglobulin recipients	DRB1*01	Viral clearance	27.4 vs. 7.1	OR 4.9
	73 RNA –				
	84 RNA +				
Thursz 1999 [222]	Cohort 1 Europeans	DRB1*0301	Viral	29.4 vs. 17.6	OR 1.94
	85 RNA –	DRB1*1101	clearance	30.6 vs. 17.1	OR 2.14
	170 RNA +	DRB1*1201	Viral	5.9 vs. 0.6	OR 10.56
		DQB1*0301	clearance	45.9 vs. 27.6	OR 2.22
		DRB1*0701	Viral	17.6 vs. 30.6	OR 0.49
		DRB1*1501	clearance	9.4 vs. 19.4	OR 0.43
		DRB4*0101	Viral	28.2 vs. 48.2	OR 0.42
	Cohort 2 United Kingdom Look back	DRB1*1101	clearance	24.6 vs. 9.9	OR 2.97
		DQB1*0301	Viral	43.9 vs. 24.3	OR 2.43
	57 RNA –	DRB1*0701	persistence	12.2 vs. 26.9	OR 0.38
	152 RNA +	DRB4*0101	Viral	31.5 vs. 46.1	OR 0.54
			persistence		
			Viral		
			persistence		
			Viral		
			clearance		
			Viral		
			clearance		
			Viral		
			persistence		
			Viral		
			persistence		
Alric 2000 [223]	French Caucasian	DRB1*1101	Viral	33.8 vs. 14.7	Pc=0.012
	63 RNA –	DQB1*0301	clearance	64.4 vs. 28.6	Pc=0.003
	282 RNA +	DQB1*02	Viral	25.4 vs. 49	Pc=0.048
			clearance		
			Viral		
			persistence		
Fanning 2000 [224]	Irish female anti-D IgG recipients	DRB1*01	Viral clearance	22 vs. 9	RR 0.37 ?Pc<0.05
	84 RNA –	DRB1*0701 in the absence of		20 vs. 0	? Pc<0.05
	72 RNA +	DQB1*0501	Viral		
			persistence		

Table 1 *(Continued)*

Reference	Population studied	Alleles or haploptype	Association	Effect size (Percentage RNA negative vs. RNA positive)	OR, RR or Pc
McKiernan 2000 [225]	Irish female anti-D IgG recipients 95 RNA – 148 RNA +	DRB1*0101 DQB1*0501 (in linkage) DRB1*03011 DQB1*0201 (in linkage)	Viral clearance Viral clearance Viral persistence Viral persistence	32.3 vs. 8.8 36.8 vs. 14.2 16.7 vs. 41.5 15.8 vs. 42.6	Pc<0.0005 Pc=0.002 Pc=0.001 Pc=0.0004
Vejbaesya 2000 [226]	Thai 43 RNA – 57 RNA +	DRB1*0301 DQB1*0201 DRB1*0301-DQA1*0501-DQB1*0201	Viral persistence Viral persistence Viral persistence	0 vs. 21.1. 4.6 vs. 28.1 NS	RR 1.96 Pc=0.04 P=0.002
Barrett 2001 [227]	Irish female anti-D IgG recipients 87 PCR + 68 PCR –	DRB1*01	Viral clearance	28.8 vs. 8.7	Pc=0.036
Thio 2001 [228]	American IDU and haemophiliac cohorts 200 RNA – 374 RNA +	DQB1*0301 DRB1*0101 DQB1*0501-DRB1*0101 (The latter associations not found in black subjects)	Viral clearance Viral clearance Viral clearance Viral clearance	24.5 vs. 18.9 10.1 vs. 4.7 9.9 vs. 4.8	OR 0.72 OR 0.45 OR 0.48
Thio 2002 [205]	American IDU and haemophiliac cohorts 231 RNA – 444 RNA +	HLA-A*1101 HLA-B*57 HLA-Cw*0102 HLA-A*2301 HLA*Cw*04	Viral clearance Viral clearance Viral clearance Viral clearance Viral persistence Viral persistence	5.3 vs. 2.8 7.3 vs. 4.6 3.7 vs. 1.7 3.9 vs. 6.3 9.6 vs. 15.3	OR 0.49 OR 0.62 OR 0.43 OR 1.78 OR 1.78
Fanning 2004 [206]	Irish (mainly female anti-D IgG recipients) 86 RNA – 139 RNA +	HLA-Cw*04	Viral persistence	4.6 vs. 17.3	Pc=0.036
Khakoo 2004 [85]	USA and UK IDUs, haemophiliacs and clinic subjects 685 RNA + 352 RNA –	HLA-C1C1 HLA-C2C2 (represents two HLA group 1 or 2 alleles)	Viral clearance Viral persistence	37.5 vs. 29.9 14.5 vs. 20.2	1.4 0.67
McKiernan 2004 [207,225]	Irish female anti-D IgG recipients 148 RNA + 95 RNA –	HLA-A*03 HLA-B*27 DRB1*0101 DRB1*0401 DRB1*15	Viral clearance Viral clearance Viral clearance Viral clearance Viral clearance	39.5 vs. 19.1 14.0 vs. 2.1 32.6 vs. 8.5 29.1 vs. 15.6	2.43 7.99 4.71 4.12 2.2

OR: odds ratio; NS: not stated; RR relative risk; Pc: P value corrected for multiple comparisons.

group has demonstrated reduced IFN-γ production in African Americans with acute HCV, suggesting a possible mechanism for the differences in outcome [200].

The most frequently studied genetic associations with effective clearance of HCV infection have been the genes of the major histocompatibility complex (MHC). The outcome of HCV infection may be related to genetically determined variations in the efficiency of presentation of viral peptides to T cell receptors on CD4 lymphocytes, which is governed by human leukocyte antigen (HLA) class II molecules. Similarly, the role of HLA class I molecules may by important as they present antigens to CD8 lymphocytes. The published studies examining HLA associations with viral clearance using molecular typing methods are summarized in Table 1.

The most common association with clearance after primary infection has been with the class II alleles, DQB1*0301 and DRB1*1101 which are in close linkage disequilibrium. A meta-analysis of published studies in this area found that these two alleles were associated with viral clearance [201]. There is experimental evidence that immunodominant HCV epitopes are presented by DRB1*1101[202] and that T cell lines recognize HCV peptides presented by DQB1*0301 [203]. Similarly, another allele that has been associated with HCV clearance, DRB1*0101 was associated with a greater magnitude and broader range of epitope responses in an HCV-specific T cell proliferation assay [204].

By contrast, only very few class I HLA allele associations have been described in association with clearance of HCV viremia. Two groups have demonstrated an association of viral persistence with the presence of HLA-Cw*04 [205, 206]. A potential mechanism for the association between HLA-C alleles and persistence has been elucidated with the recognition that these class I alleles interact with inhibitory NK cell receptors [85]. This interaction is likely to play a significant role in the innate immune response against HCV infection when low dose innocula are encountered (such as in IDU). The presence of the HLA-B27 allele has also been associated with viral clearance [207] and a functional mechanism for this effect has been reported with the recognition of an HLA-B27 restricted CD8 epitope [208].

Other immunogenetic polymorphisms have recently been reported to influence the outcome of HCV infection. They include polymorphisms in chemokine receptors, such as a single nucleotide polymorphism (SNP) of chemokine receptor 2 (CCR2) which was under-represented in people who had cleared HCV infection [209] and polymorphisms of the promoter region of the interleukin-10 (IL-10) gene with the IL-10-592 AA genotype associated with self limiting infection, and the IL-10-1082 GG genotype associated with persistent infection [210, 211]. Another study demonstrated an association between the IL-10 ATA haplotype (IL-10-1082A, -819T, -592A) and spontaneous HCV clearance [211]. The authors postulated that the reduced production of IL-10 associated with this haplotype may result in a Th1-polarised CD4+ T cell response, which may be associated with enhanced viral elimination.

Polymorphisms of ISGs have also been found to be associated with HCV clearance [212]. A polymorphism with the GG genotype at position 88 in the myxovirus resistance-1 (MxA) gene, and in the 3' untranslated region of the 2-5-oligoadenylate synthetase-1 (OAS-1) gene, was less common in people with resolved HCV infection. A polymorphism in the promoter region of the PKR gene has been associated with self-limiting HCV infection [213]. An association between inducible nitric oxygen synthase gene haplotypes and HCV clearance has also been reported [214]. The functional significance of these variants has not yet been examined. The presence of polymorphisms of the transforming growth factor (TGF)-β1 gene promoter that reduce expression of TGF-β1 are also associated with increased rates of clearance of HCV infection [215]. TGF-β1 suppresses the proliferation and cytotoxicity of NK cells amongst other roles. This finding therefore adds to other data potentially implicating NK cell activity in HCV clearance [85, 205].

Replication of the disease association findings (other than HLA) in independent cohorts across different ethnic groups is required to resolve the preliminary findings. Subsequent studies will then be required to verify the functional significance of the polymorphisms in the candidate genes in relation to HCV infection.

Conclusion

The detailed mechanisms allowing host clearance of viremia in acute HCV infection remain only partially defined. Strong evidence points to aspects of the cellular immune response as critical to the outcome (see Fig. 4 for summary). The priorities in further research include application of improved assay systems to longitudinally collected samples from subjects with acute HCV to resolve factors associated with viral clearance, including: i) the role of neutralizing antibodies; ii) the combined influences of the number and function of specific CD4+ and CD8+ T cell sub-populations; and iii) the pattern of anti-viral cytokines, including some ISGs. These studies, in

combination with detection of genetic polymorphisms associated with clearance may allow reliable prediction of the probability of clearance with a high degree of accuracy during early infection, and hence guide individually-tailored antiviral treatments. Better understanding of the immunopathogenesis of primary HCV infection will also guide HCV vaccine development by identification of potential immunodominant viral proteins and cross-reactive immune responses targeting epitopes associated with these proteins.

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