Immunopathogenesis in hepatitis C virus cirrhosis

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ABSTRACT

HCV (hepatitis C virus) has a high propensity to persist and to cause chronic hepatitis C, eventually leading to cirrhosis. Since HCV itself is not cytopathic, liver damage in chronic hepatitis C is commonly attributed to immune-mediated mechanisms. HCV proteins interact with several pathways in the host’s immune response and disrupt pathogen-associated pattern recognition pathways, interfere with cellular immunoregulation via CD81 binding and subvert the activity of NK (natural killer) cells as well as CD4+ and CD8+ T-cells. Finally, HCV-specific T-cells become increasingly unresponsive and apparently disappear, owing to several possible mechanisms, such as escape mutations in critical viral epitopes, lack of sufficient help, clonal anergy or expansion of regulatory T-cells. The role of neutralizing antibodies remains uncertain, although it is still possible that humoral immunity contributes to bystander damage of virally coated cells via antibody-dependent cellular cytotoxicity. Cytotoxic lymphocytes kill HCV-infected cells via the perforin/granzyme pathway, but also release Fas ligand and inflammatory cytokines such as IFNγ (interferon γ). Release of soluble effector molecules helps to control HCV infection, but may also destroy uninfected liver cells and can attract further lymphocytes without HCV specificity to invade the liver. Bystander damage of these non-specific inflammatory cells will expand the tissue damage triggered by HCV infection and ultimately activate fibrogenesis. A clear understanding of these processes will eventually help to develop novel treatment strategies for HCV liver disease, independent from direct inhibition of HCV replication.

DEFINING A ROLE FOR THE IMMUNE SYSTEM IN THE PATHOGENESIS OF CHRONIC HCV (HEPATITIS C VIRUS) INFECTION

Infection with HCV has been increasingly recognized to constitute a major health problem, because a substantial proportion of patients (50–80 %) will develop persistent viraemia following acute infection. Chronic HCV infection is accompanied by variable degrees of hepatic inflammation, damage to the liver and progressive fibrosis with an increased risk for progression towards cirrhosis and hepatocellular carcinoma. A recent Canadian study [1] estimated lifetime HCV-associated mortality to be approx. 1 in 8 and a much larger number of patients (1 in 4) has been estimated to develop cirrhosis.

Failure to generate sufficiently effective immune responses during the acute phase of infection is considered a

Key words: antibody, cirrhosis, hepatitis C virus (HCV), immune response, liver damage, T-cell.

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; CCR, CC chemokine receptor; CMV, cytomegalovirus; CTL, cytolytic T-lymphocyte; DC, dendritic cell; ds-RNA, double-stranded RNA; HCV, hepatitis C virus; IFN, interferon; IKK-ε, I-κB kinase; IL, interleukin; IP-10, 10 kDa IFNγ -inducible protein; IRF3, IFN regulatory factor 3; LCMV, lymphocytic choriomeningitis virus; MMP2, matrix metalloproteinase 2; NK, natural killer; NK-κB, nuclear factor κB; PAMP, pathogen-associated molecular pattern; RIG-I, retinoic-acid-inducible gene-I; TGFβ, transforming growth factor β; TLR, Toll-like receptor; TNF, tumour necrosis factor; TNFR, TNF receptor; TANK, TNFR-associated factor family member associated NF-κB activator; TBK1, TANK-binding kinase 1; TRAIL, TNF-related apoptosis-inducing ligand; Treg, regulatory T-cell; TRIF, TIR domain-containing adaptor protein inducing IFNβ.

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key factor in developing chronic hepatitis C [2,3], and it has become quite clear meanwhile that effective elimination of HCV infection requires the co-ordinated function of multiple arms both of the innate immune system [IFNs (interferons), NK (natural killer) cells and NK T-cells, γδ T-cells and antigen-presenting cells, including several types of DCs (dendritic cells)] and the adaptive immune system (neutralizing antibodies and CD4+ and CD8+ T-cells). In hepatitis C, there is circumstantial evidence suggesting that activation of the immune response is also a pivotal factor in the pathogenetic processes leading to progressive tissue injury and ultimately cirrhosis. Studies in various expression systems (cell culture or transgenic mice) indicate that HCV is not directly cytopathic and viral replication may occur in the absence of a detectable inflammatory reaction [4,5]. On the other hand, liver cell damage is associated with the presence of an intrahepatic inflammatory infiltrate. Of note, hepatocellular damage coincides with the onset of an immune response during acute infection, but not with that of viral replication [6]. Moreover, transient amelioration of inflammatory activity and hepatocellular damage, together with increased HCV viraemia, have been reported under immunosuppressive therapy [7], whereas inflammatory exacerbation of hepatitis C can occur when immunosuppressive therapy is terminated [8,9]. Taken together, the immune response is a double-edged sword in chronic hepatitis C. Despite failure to achieve viral elimination during the acute phase of HCV infection, immune effector mechanisms are sufficiently effective to mediate liver damage in chronic HCV infection. On the other hand, the immune system may still contribute importantly to the control of chronic infection preventing excessive levels of HCV replication.

The histological hallmark of chronic hepatitis C is a necro-inflammatroy process characterized by interface hepatitis, bridging necrosis, and the formation of portal tract to central vein and portal tract to portal tract fibrous septa as well as septa blind-ending in the parenchyma. Further pathomorphological features comprise cell shrinkage, fragmentation of the cell nucleus and the presence of acidophilic Councilman bodies as well as lobular dropout of liver cells. This type of lesion suggests apoptosis as an important step in the pathogenesis of chronic hepatitis C. However, the relative contribution of either apoptosis, which does not release aminotransferases, and necrosis to chronic hepatitis have not yet been clearly determined. The variable contribution of apoptosis in individual patients, which does not cause release of intracytoplasmic constituents into the circulation, compared with necrosis probably explains why, in some patients, we find progressive liver damage and cirrhosis despite persistently normal aminotransferases [10], and why surrogate markers of inflammation correlate poorly with intrahepatic fibrosis in chronic hepatitis C [11].

Since HCV itself does also not appear to predict the progression of fibrosis, immune-mediated inflammatory damage is the key process in the pathogenesis of chronic hepatitis C. However, disease progression is slow over several decades and is associated with mild intrahepatic inflammatory infiltrates in the vast majority of patients [12]. Finally, the rate of fibrosis progression can be importantly modified by external factors, such as excessive concomitant alcohol intake or infection with other viruses.

In this review, we discuss current concepts of HCV-related immunopathogenesis to illustrate how innate and adaptive immunity interact with viral components and ultimately shape the outcome of HCV infection. We also focus on the mechanisms of how the immune response contributes to liver damage promoting fibrosis progression towards cirrhosis.

**HCV INTERACTIONS WITH THE HOST IMMUNE SYSTEM**

**Interaction with TLRs (Toll-like receptors) and other pathogen-specific pattern-recognition receptors**

Hepatitis C virus is a positive sense RNA virus classified as a hepacivirus in the Flaviviridae family. Its viral genome encodes a single polyprotein of 3000 amino acids in length, which is cleaved into three structural proteins (HCV core, and envelopes E1 and E2), six non-structural proteins (HCV NS2, NS3, NS4A, NS4B, NS5A and NS5B) needed for viral replication and the HCV p7 protein, the function of which has not yet been determined. The various viral components (RNA, viral proteins and intact virions) can be recognized by the immune system as specific foreign antigens, but may also activate innate immunity via distinct cytoplasmic and cell-surface receptors, which are triggered by PAMPs (pathogen-associated molecular patterns) and give rise to general alarm signals. HCV protease NS3/4A may importantly interfere with signalling in these activation pathways.

For instance, TLR3 and TLR7 on the cell surface or within membrane-bound cytosolic vesicles may sense the presence of virus-derived ds-RNA (double-stranded RNA) and ss-RNA (single-stranded RNA) species respectively [13,14]. Intracellularly, PAMP signalling via TLR3 involves recruitment of the TRIF (TIR domain-containing adaptor protein inducing IFNβ) adaptor protein and downstream phosphorylation and activation of IRF3 (IFN regulatory factor 3) via the protein kinases IKK-ε (IκB kinase-ε) and TBK1 (TANK-binding kinase 1, where TANK is TNFR [TNF (tumour necrosis factor) receptor]-associated factor family member associated NF-κB (nuclear factor κB) activator) [15–19]. TRIF
also directs MyD88-independent activation of the transcription factor NF-κB [14]. Ultimately, activated transcription factors IRF3 and NF-κB up-regulate the production of type 1 IFNs [15] and thus induce enhanced expression of IFN-inducible genes, most of which exert important antiviral and immunoregulatory functions. Importantly, type 1 IFNs up-regulate MHC class I molecules, the central recognition element of cell-mediated immune responses, and stimulate maturation and differentiation of cytotoxic effector cells [19]. NF-κB is also involved in regulating the expression of chemokines and inflammatory cytokines, which, in parallel with type 1 IFNs, mediate the inflammatory response to HCV. Finally, NF-κB is involved in the regulation of hepatocellular apoptosis [20].

The product of RIG-I (retinoic-acid-inducible gene-I) is a cytoplasmic receptor for ds-RNA [21], which is formed as an intermediate during replication of RNA viruses. RIG-I contains two N-terminal CARDs (caspase-recruitment domains), which interact with the adapter protein Cardif [also termed IPS-1 (IFN promoter-stimulator-1), MAVS (mitochondrial anti-viral signalling protein) or VISA (virus-induced signalling adapter)] initiating downstream phosphorylation of protein kinases IKK-ε and TBK1. Thus signal transduction in the RIG-I and TLR3 pathways converge at activating shared signal transduction proteins, leading to induction of antiviral and immunoregulatory genes via transcription factors IRF3 and NF-κB [22–25].

HCV protease NS3/NS4A can cleave both the adaptor proteins Cardif [21] and TRIF [26] as part of the viral defence against the host immune response, thus disrupting signal transmission of PAMP-induced alarm signals. Highly complex interactions between HCV and immunostimulatory pathways are exemplified further by multiple observations in which HCV could inhibit both IFN signalling via the JAK (Janus kinase)/STAT (signal transducer and activator of transcription) pathway and IFN-inducible proteins by a variety of different mechanisms [27]. In this context induction of IL (interleukin) 8 (CXCL8) by the HCV NS5A protein is remarkable [28], because it inhibits IFN activity in vitro [29] and in vivo [30] and also acts as a pro-inflammatory and chemotactic cytokine to attract inflammatory cells independently from antigen recognition. Together with other growth factors, IL8 also directly activates mesenchymal cells and extracellular matrix production.

Distinct up-regulation of TLRs has been reported in specimens from patients with HCV infection [31,32]. In particular, NS5A induces expression of TLR4 on B-lymphocytes and leads to enhanced secretion of IFNα and IL6 [33]. Moreover, HCV core and NS3 proteins trigger TLR2 on monocytes and macrophages to induce secretion of the inflammatory cytokines IL1, IL6, IL8 and TNF [34]. Although PAMP-dependent pathways are part of the innate immune responses against HCV, up-regulation of TLRs and stimulation of pro-inflammatory cytokines are likely to contribute to the pro-inflammatory environment in chronic HCV infection. At this stage, antiviral cellular immunity is substantially impaired, but continued activation of cytokine secretion and HCV non-specific effector pathways lead to continued hepatocyte damage, fibrogenesis and malignant transformation.

Interactions of HCV with the tetraspanin CD81

Although the mechanism of HCV cellular entry is currently not understood, several surface molecules have emerged as potential receptors for HCV. Of note, HCV E2 and the E2/E1 envelope complex expressed on HCV particles have been shown to bind to CD81 [35,36]. CD81 is a 26 kDa protein member of the tetraspanin superfamily, which is expressed on most human cells and can be up-regulated in response to cellular activation [37]. A shared property of tetraspanins, including CD81, is their propensity to associate with other membrane proteins to form signal transduction complexes, the partners of which may vary depending on the cell type [38]. Thus binding of HCV E2 can induce a variety of different functional changes depending on the type of cells involved. For instance, binding of HCV E2 to CD81 stimulates the proliferation of naïve (CD27−) B-lymphocytes [39]. CD81 triggering on NK cells has been reported to alter their functions [40,41]. In vitro, ligation of CD81 on NK cells by anti-CD81 or immobilized recombinant HCV E2 protein blocked the release of cytolytic granules, IFNγ production and proliferative response to IL2. Unlike NK cells, in vitro stimulation of intrahepatic γδ T-cell lines by cross-linking CD81 induced the release of IFNγ and TNFα [42]. Increased proportions of γδ T-cells have been specifically reported in HCV-infected livers [43,44], in particular those with high necro-inflammatory activity [44,45]. It is noteworthy in this context that CD81 cross-linking on γδ T-cells triggers secretion of inflammatory cytokines without the need for T-cell-receptor activation. Thus E2/CD81 interactions between HCV and γδ T-cells may contribute to hepatic inflammation via a process that lacks T-cell-receptor specificity. Cross-linking of CD81 on conventional αβ T-lymphocytes has co-stimulatory activity and can likewise lead to non-specific cytokine production and T-cell proliferation [46].

E2/CD81 interactions stimulate RANTES (regulated upon activation, normal T-cell expressed and secreted) secretion in CD8+ T-cells and induce the subsequent internalization of CCR5 (CC chemokine receptor) 5, thus altering migration of immunocompetent cells [47,48]. In addition, direct stimulation of CD81 on DCs disrupts their migratory response to CCL21, a chemokine which normally redirects migration of activated DCs back into
lymphoid tissue to enhance effective immune activation [49]. Interruption of the physiological circulation of DCs between the liver, the site of infection in chronic hepatitis C, and lymphoid tissue is likely to result in trapping of activated DCs in the liver and may account for the reported changes in the number and function of DCs in peripheral blood and the liver [50–54]. In vitro binding of the HCV E2 envelope protein to T-lymphocytes inhibits their chemokine secretion and migration induced via LFA1 (leucocyte function antigen 1) integrin stimulation [55].

Binding of HCV E2 to CD81 may directly act on hepatic stellate cells, the major effector cell type of hepatic fibrosis, to secrete MMP2 (matrix metalloproteinase 2) [56]. The putative implications of this process are that, once secreted, MMP2 disrupts the normal basement membrane, and then these lesions activate hepatic stellate cells. In addition, degradation of extracellular matrix proteins by MMP2 may facilitate inflammatory cells to penetrate to the sites of hepatic injury. Beyond HCV E2, other HCV structural (e.g. core) and non-structural (e.g. NS5A) proteins are also likely to contribute directly to the regulation of hepatic fibrogenesis in chronic hepatitis C [57,58].

Binding of HCV envelope proteins and presumably also intact virions to surface proteins on hepatocytes may ‘label’ these cells for recognition by anti-envelope antibodies. Anti-E2 antibodies are detected readily in patients with chronic hepatitis C [59] and, thus, hepatocytes bearing E2-anti-E2 complexes on their surface may become targets of ADCC (antibody-dependent cellular cytotoxicity) [60]. Antibody binding can occur independently from direct HCV infection of the cell. Therefore ADCC in chronic hepatitis C is a probable process also contributing to innocent bystander lysis of liver cells.

Taken together, the recent years of research have provided accumulating evidence that HCV interacts with the host’s immune defence via multiple different cellular and molecular pathways. It can be anticipated that further effects of HCV interacting with immunoregulatory molecules, such as DC-SIGN (DC-specific intercellular adhesion molecule-3-grabbing non-integrin) and its related molecule CD209L [also know as L-SIGN (liver/lymph-node-specific intercellular adhesion molecule-3-grabbing non-integrin)], will become unravelled in the near future [61–65]. In summary, all these interactions are increasingly complex and occasionally may result in effects counteracting each other in individual patients. Thus it remains difficult to assess from the experimental data the net outcome of HCV-host interactions. However, it is an intriguing speculation that HCV-host interactions (i) blunt the directly antiviral pathways, (ii) enhance and broaden the immune response by triggering non-specific inflammatory and cytolytic mechanisms, and (iii) create an intrahepatic milieu facilitating activation of hepatic stellate cells and fibrogenesis.

**IMMUNE RESPONSE IN CHRONIC HCV INFECTION**

**Role of NK cells**

NK cells play an important part in the first line of defence against viral infections. They rapidly recognize and lyse infected cells, and also secrete inflammatory cytokines which inhibit viral replication and exert immunoregulatory functions providing a pivotal link between innate and adaptive immunity. Epidemiological studies in HCV infection indicate that NK cells constitute a critical element in the initial antiviral response and contribute to HCV elimination [66]. However, analysis of NK cell numbers and functional ex vivo studies have yielded conflicting results on the potential roles of NK cells in chronic hepatitis C [67–69].

Activity of NK cells is regulated by the complex interplay of activating and inhibitory NK receptors with their respective ligands comprising, among others, the KIR (killer Ig-like receptor) family (ligands HLA-A, -B and -C), the CD94–NKG2A/C complex (ligand HLA-E), NKG2D (ligands MIC-A and MIC-B) and the natural cytotoxicity receptors Nkp30, Nkp44 and Nkp46 [70]. In addition, part of these receptors also exert immunoregulatory functions in subsets of T-lymphocytes. Binding of HCV peptides to HLA-E and subsequent up-regulation of HLA-E expression has recently been described in vitro [71]. These findings correspond to up-regulated HLA-E expression on a great variety of different hepatic cells in biopsies from patients with chronic hepatitis C as well as altered NK cell functions mediated via interactions of HLA-E with CD94–NKG2A [72,73]. In these studies, NK cells exerted reduced levels of cytolytic activity and instead secreted abundant amounts of IL10 and TGFβ (transforming growth factor β). In addition, cross-talk between NK cells and DCs to drive immune responses towards generating TH1-polarized CD4+ T-cells was also markedly impaired by CD94–NKG2A-mediated interactions. Furthermore, in chronic hepatitis C, expression of the inhibitory receptor CD94–NKG2A has been found to be markedly up-regulated on both NK cells and cytolytic CD8+ T-lymphocytes, whereas expression of activating natural cytotoxicity receptors Nkp30 and Nkp46 was reduced [73]. These findings have important implications. Failure to generate sufficiently effective cytolytic activity and TH1-polarized antiviral CD4+ T-cell responses probably contribute importantly to viral persistence in chronic hepatitis C. Beyond that, down-regulated cytolytic activity of NKs in chronic hepatitis C and their altered cytokine response may directly enhance fibrosis progression, because IL10 and...
TGFβ stimulate proliferation and extracellular matrix deposition in hepatic stellate cells [74] and experimental models suggest that NK cells regulate liver fibrosis by killing activated hepatic stellate cells [75,76].

**Humoral immunity in chronic hepatitis C**

Antiviral antibodies usually become detectable within several weeks after acute HCV infection and constitute an important tool for the diagnosis of HCV infection. The role of naturally acquired HCV antibodies in contributing to protection is discussed controversially because the presence of anti-HCV cannot prevent re-infection [77,78]. Moreover, in selected cases, HCV infection can resolve without developing HCV-specific antibodies [79,80]. On the other hand, adaptive sequence changes in the hypervariable HCV E2 region 1, the putative target of neutralizing antibodies, have been described in acutely HCV-infected individuals and were found to be correlated with the outcome of infection [81]. Importantly, these sequence changes occurred at the time of anti-HCV seroconversion. Thus they have been interpreted to indicate that HCV adapts to the immune selection pressure exerted by natural HCV antibodies via escape mutations. A protective contribution of HCV-specific antibodies in HCV infection is supported further by the observation that HCV pre-treatment in vitro with antibodies prevented infectivity in the chimpanzee model [82,83]. Lentiviral pseudotype particles bearing native HCV envelope glycoproteins on the surface have recently become available and have been used as tools to demonstrate cross-genotype neutralization of HCV by antibodies in sera from patients with chronic hepatitis C [84–86]. Such neutralizing antibodies could exert protective immunity [87], but occurred only infrequently in patients with self-limited HCV infection [84–86]. Finally, the antibody response and its effect on the evolution of HCV during chronic infection has also recently been linked to the level of liver injury [88], and anti-E2 antibodies with the capability to mediate liver damage via ADCC occur mainly only after a prolonged HCV infection [60]. Thus the precise contributions of natural HCV antibodies to viral elimination compared with liver damage and fibrosis progression still remain to be determined.

**Antiviral T-cell-mediated immunity in chronic hepatitis C**

T-cell-mediated immunity involves CD8+ CTLs (cytolytic T-lymphocytes), which recognize linear peptides of 8–11 amino acids in length bound to self-HLA class I molecules, and CD4+ T-helper lymphocytes, which can recognize longer viral peptides bound to class II molecules. Cell-mediated immunity is considered the key mechanism for resolution of primary HCV infection [89]. The spectrum of T-helper cells isolated from peripheral blood and liver in HCV-infected patients indicates a multi-specific response targeted at several conserved and variable epitopes which are presented via distinct HLA alleles both for CTLs [80–102] and T-helper cells [103–112]. In acute hepatitis C, strong HCV-specific CTLs [113,114] and TH1-type CD4+ T-helper cell responses [106,115] have consistently been reported to be closely associated with a self-limited course of HCV infection. Moreover, several groups have reported an inverse relationship between the strength of the CTL response and HCV viral loads [116–118], suggesting further that in principle it is possible for cellular immunity to control HCV infection [119]. Of note, cellular immunity is maintained long-term in those individuals who have successfully eliminated their HCV infection [120]. It has now become clear that a substantial proportion of individuals who eventually develop chronic hepatitis C generate HCV-specific CD4+ and CD8+ T-cell responses during the early acute phase of their infection and transiently gain control over HCV replication [114,121–124]. However, these early T-cell responses decline to almost undetectable levels thereafter and initial control over HCV infection is lost. If present, HCV-specific CD4+ and CD8+ T-cells are detected at only low frequency in peripheral blood, although they are somewhat enriched in the liver [125,126]. The exact state of HCV-specific CD4+ and CD8+ T-cells is not clear in persisting HCV infection, but T-cells show a couple of characteristic abnormalities in phenotype and function. HCV-specific CD8+ T-cells predominantly carry a CCR7high/perforinlow phenotype and fail to secrete IFN-γ and to exert significant ex vivo killing [113,127,128]; CD4+ T-cells have reduced HCV-specific proliferation and low production of IL2 and IFNγ [129–132]. Instead, IL10 and TGFβ are preferentially induced, indicating that CD4+ T-helper cell differentiation has become biased towards secretion of anti-inflammatory cytokines [130,132–135]. T-cell abnormalities in chronic hepatitis C are potentially reversible, since addition of IL2 or stimulation via TLR2 can restore impaired in vitro T-cell proliferative responses [136–138], and therapeutic restoration of an antiviral TH1 response supports sustained clearance of HCV infection [139]. Thus understanding which mechanisms down-regulate HCV-specific CD8+ and CD4+ T-cell responses has become a key issue in the immunopathogenesis of HCV infection, and appearance of T-cell escape mutations, lack of sufficient T-cell help, T-cell exhaustion and generation of so-called Tregs (regulatory T-cells), which exert inhibitory effects, have been proposed as possible causes for T-cell dysfunction in chronic hepatitis C.

In common with other RNA viruses, HCV replication by an error-prone RNA-dependent RNA polymerase generates genomic diversity with the potential to generate minor viral variants that can evade immune recognition.
Epitopes may be lost, because substitutions of amino acids alter proteasomal processing, binding to MHC molecules or CTL recognition of variant peptides [140]. Despite the continued presence of large numbers of viral epitopes in persistent HCV infection, new epitope variants selected by immune pressure apparently do not elicit strong CD8+ T-cell responses, either because the new variant is not immunogenic or, due to a phenomenon termed the 'original antigenic sin', the immune system does not respond to an epitope which is only slightly modified, or CD4+ T-cell help is insufficient to support the induction of new CD8+ T-cell epitopes [141].

Mutations of HCV in critical CD8+ T-cell epitopes leading to escape from immune recognition have been identified only recently in longitudinal studies of acutely infected patients and in single source outbreaks [142–144]. CD8+ T-cell responses are also affected by HCV mutations which alter proteasomal processing of HCV antigens [145]. In contrast, the role of HCV escape mutations in evasion from CD4+ T-cell responses still remains elusive.

Delayed acquisition and expansion of HCV-specific CD8+ T-cell effector functions would favour accumulation of variants that can evade immune recognition. However, the observations in humans and data obtained in chimpanzees suggest that CD8+ T-cell escape mutations occur early after infection [146]. In addition, delayed acquisition of CD8+ T-cell responses of its own is not sufficient to explain escape from multi-specific CTL responses, because escape mutations are not found in all T-cell epitopes, whereas a decline in all T-cell responses is observed in chronic hepatitis C [144,147]. However, spontaneous loss of CD4+ T-cell help also has to be taken into account and may undermine the effectiveness of HCV-specific CTL responses [123]. Thus CTL functions might become impaired to an extent that they can no longer mediate viral clearance, but still exert sufficient pressure to select HCV variants. This concept is supported by experiments in HCV-immune chimpanzees, where depletion of CD4+ T-cells resulted in persistent HCV infection and the appearance of multiple CTL escape mutations upon re-challenge with the same HCV strain [148]. However, it remains unclear which mechanisms cause loss of CD4+ T-cell function in persistent HCV infection, because the vast majority of HCV-specific CD8+ T-cells are not deleted and their functionality can be restored in vitro [107,110,136]. Moreover, in the chimpanzee model of chronic hepatitis C, DCs did not reveal defects in phenotype or function, making deficient priming of CD4+ T-cells by antigen-presenting cells a less likely possibility [149].

Exhaustion of T-cells (clonal deletion/energy) is an alternative concept to explain altered T-cell responses in chronic virus infections, which has been derived from experiments in the murine model of LCMV (lymphocytic choriomeningitis virus) infection [150]. In this model, persistent high level viraemia can be established in susceptible mouse strains or by infection with more pathogenic virus variants. Initially, mice develop a robust T-cell response but fail to eliminate the virus and, subsequently, exhibit a gradual decline and ultimately complete loss of CD8+ and CD4+ T-cell responses. Further research has suggested that T-cell exhaustion represents a continuum of altered responses giving rise also to 'partial exhaustion', characterized by the progressive loss of IL2 and IFN secretion as well as reduced proliferative capacity and impaired cytotoxicity, before full clonal deletion occurs [151]. CD8+ T-cells in chronic hepatitis C have been shown to be functionally impaired or anergic [127,128,152] and, consistent with this loss of function, exhibit phenotypic changes indicating early stages of differentiation [153]. In line with the LCMV model, anergy of CD8+ T-cells in chronic hepatitis C is associated with declining virus-specific CD4+ responses [154,155], but it remains unclear if HCV-specific CD4+ T-cells, which may become undetectable in chronic hepatitis C [110], are actually deleted. Full exhaustion with deletion of antigen-specific CD8+ T-cells, however, obviously does not occur, since CD8+ T-cell responses can be rescued in vitro with IL2 and antigen [99]. Although evolution of HCV-specific CD8+ and CD4+ T-cell responses in chronic hepatitis C match several features of the T-cell exhaustion model, the significance of these analogies remains uncertain, since impaired functions of virus-specific CD8+ T-cells were also observed after resolution of HCV infection [152]. Moreover, in chronic hepatitis C, changes in phenotype were likewise observed in CMV (cytomegalovirus)-specific CD8+ T-cells, which, however, did not reveal relevant dysfunction [156]. Finally, although PD1/PD1 ligand signalling has been proposed as a mechanism for anergy in the LCMV model [157], the cellular and molecular mechanisms underlying putative T-cell exhaustion in chronic hepatitis C are not understood.

Murine models have also provided experimental evidence for naturally occurring CD4+CD25+Foxp3+ Tregs and several subsets of induced suppressor T-cells as key players in the regulatory network of the immune system [158]. Tregs actively control induction and effector functions of other immune cells by suppressing their functional activity via contact-dependent mechanisms as well as by secreting immunosuppressive cytokines, such as IL10 and TGFβ. CD8+ T-cell lines producing IL10 in response to HCV antigens were the first evidence for antigen-specific MHC class I-restricted regulatory cells which could down-regulate antiviral responses [159]. Recently, these initial findings were confirmed by the observation that CD8+ T-cells exist in the livers of patients with chronic hepatitis C, which secrete IL10 and suppress in vitro proliferation of liver-derived T-cells in an HCV-specific manner [160]. Beyond that, numbers of CD4+ Tregs were found to be
increased in the peripheral blood of patients with chronic hepatitis C, and the depletion of CD4+CD25+ T-cells was associated with increased numbers and function of CD8+ T-cells in in vitro assays [161–164]. The various types of Tregs may reduce inflammatory activity and are considered to contribute importantly to preventing immune-mediated pathology in chronic hepatitis C. On the other hand, a critical issue in the interpretation of these data is that hyporesponsiveness in chronic hepatitis C is HCV-specific but leaves other responses intact, whereas CD4+CD25+ Tregs in peripheral blood also inhibited responses to CMV and Epstein–Barr and influenza viruses [163,164]. Although preferential compartmentalization of HCV-specific T-cells to the liver may concentrate Tregs at the site of HCV infection, further studies are needed to clarify whether intrahepatic Tregs protect against immunopathology and whether they contribute to the observed loss of HCV-specific CD8+ and CD4+ T-cell responses seen in chronic hepatitis C.

Whatever mechanisms may contribute in detail, it is an intriguing working hypothesis that, in summary, HCV persistence is initiated by multi-faceted viral effects which interfere with innate immunity and result in deficient CD4+ T-cell help. Depending on the severity of CD4+ T-cell dysfunction, HCV-specific CD8+ T-cells either succeed or fail to control the infection. In the case of chronic hepatitis C, CD8+ T-cells persist in the liver and quite probably contribute to continued liver damage.

**THE IMMUNE RESPONSE AS A CAUSE OF LIVER DAMAGE IN CHRONIC HEPATITIS C**

A prominent lymphocytic infiltrate is the histological hallmark of chronic hepatitis C. Although in the acute phase of infection intrahepatic lymphocytes are critical for viral elimination, their continued presence in chronic hepatitis C may result in progressive liver damage. A statistical relationship between elevated aminotransferases and the presence of intrahepatic CD8+ T-cells was claimed by some studies [165,166], but in general such associations have not been a consistent finding. Moreover, hepatic infiltrates in chronic hepatitis C are quite probably composed predominantly of HCV non-specific cells [167]. In addition, HCV-specific CTLs may exert significant bystander killing via perforin, Fas/Fas ligand and TNF pathways [168,169], and particularly severe liver pathology was observed in patients where heterologous immunity was encountered due to the expansion of pre-existing cross-reactive CD8+ T-cells originally targeted at other viruses [170,171].

Increased intrahepatic levels of pro-inflammatory cytokine mRNA species have been reported in chronic hepatitis C [172–174] and were apparently linked to severity of portal inflammation and liver fibrosis in some studies [174,175]. Of note, transgenic mice expressing IFNγ under the control of a liver-specific promoter developed chronic hepatitis [176], and upregulated expression of IFN-responsive genes was reported in chronic hepatitis C and was apparently correlated with the extent of liver damage [174,177]. IFN-inducible protein IP-10 (10 kDa IFNγ-inducible protein), the natural ligand of the CXCR3 chemokine receptor, is induced in hepatocytes surrounded by infiltrating lymphocytes in chronic hepatitis C [178]. Thus inflammatory cytokines in chronic hepatitis C may promote non-specific recruitment of CXCR3-expressing NK, γδ T-cells and cytolytic CD8+ T-lymphocytes by inducing enhanced expression of chemokines, such as IP-10 [179]. Beyond their role in lymphocyte recruitment, intrahepatic chemokines in chronic hepatitis C may also directly modulate hepatic fibrogenesis [180].

The granzyme B/perforin pathway is considered a major cytolytic effector mechanism of cellular immunity. Upon activation, perforin is released from the granules of cytotoxic T-cells and NK cells to form pores in the membranes of target cells facilitating entry of concomitantly secreted granzymes. Granzymes cleave caspase 8 in the cytoplasm of target cells thus activating downstream effector caspases and inducing apoptosis [181,182]. Although this mechanism is pivotal in killing virus-infected hepatocytes, its role in bystander cytolysis is less clear, because in vitro perforin-dependent lysis of bystander cells was observed only at high effector/target cell ratios [168].

Expression of Fas ligand and secretion of TNF constitute further effector arms of the cellular immune response. Triggering of Fas (CD95) or TNFR1 induces formation of so-called death-inducing signalling complexes in susceptible target cells, resulting in the activation of caspase 8. Large amounts of activated caspase 8 may directly activate downstream effector caspases and induce apoptosis. Hepatocytes represent so-called type II cells in which activation of caspase 8 alone is not sufficient to induce cell death, but amplification via the mitochondrial pathway is required. In this pathway, activated caspase 8 causes pro-apoptotic cleavage of a protein termed Bid, which induces opening of the mitochondrial permeability transition pores and release of cytochrome c into the cytoplasm [183]. Next, cytoplasmic cytochrome c binds to APAF1 (apoptotic protease-activating factor 1), generating a complex that binds and activates caspase 9, which subsequently leads to further activation of the downstream effector caspases and induction of programmed cell death. Inappropriate expression of death factors can lead to liver destruction, whereas anti-apoptotic molecules, e.g. Bcl-2, can block cytochrome c release and demonstrate protection against Fas-induced liver damage [184,185]. HCV proteins can interact with apoptotic pathways and have been demonstrated to exert...
Summary of immunopathological mechanisms putatively involved in the pathogenesis of HCV-associated cirrhosis

Left-hand panel, innate immune responses. (A) The liver is the primary site of HCV infection. In vitro evidence suggests that HCV counteracts induction of type I IFNs in hepatocytes via inhibition of TLR3 and RIG-I pathways, and also interferes with intracellular IFN signalling. Fragments of HCV or intact particles can interact with cellular receptors, such as CD81, to inhibit the function of NK cells, to alter chemokine release and to impair trafficking of DCs and T-cells. Taken together, these interactions between HCV and the host weaken innate immunity as a first line of defence against HCV infection. Insufficient innate immunity also leads to deficits in the generation of adaptive immune responses. It is probably at this early stage of HCV infection that outcome of infection is determined. Middle and right-hand panels, adaptive immune responses. (B) Studies in peripheral blood suggest that at later stages in HCV infection there is insufficient expansion and maturation of HCV-specific T-cells probably owing to insufficient help, T-cell escape mutations, clonal exhaustion and the generation and expansion of Foxp3+ Tregs. CD4+ T-cells in chronic hepatitis C show poor IL2 secretion and proliferative responses, and CD8+ T-cells exert only little cytolytic activity. Unlike T-cells in chronic hepatitis C, there is abundant proliferation of B-cells and antibody secretion. Most of these antibodies obviously do not contribute to virus neutralization. On the other hand, there is the possibility that antibodies may contribute to bystander damage via immune complexes and ADCC. (C) In chronic hepatitis C, up-regulation of HLA-E on hepatocytes and inhibitory NK receptors on NK cells and CD8+ T-cells is observed. These changes may be part of the viral defence against the host’s immune response which may preferentially protect HCV-infected cells. In addition to recruitment of virus-specific T-cells, inflammatory cells with other specificities are also recruited and can damage uninfected bystander cells. Interaction of Fas ligand released from inflammatory cells with its receptor Fas on hepatocytes is a further candidate mechanism to enhance liver damage. Finally, inflammatory cells directly activate intrahepatic fibrogenesis via release of inflammatory and myofibroblast-activating cytokines, such as TGFβ. Thus tissue damage and fibrosis in chronic hepatitis C are probably the result of long-term activation of non-specific inflammatory immune responses.

HCV infection induces TNF production in hepatocytes [197], and virus-specific CTLs have also repeatedly been shown to secrete TNF in vitro [159,168]. Moreover, plasma TNF levels were increased in HCV-infected patients, but elevated plasma levels of TNF and the corresponding soluble TNFR1 were mainly elevated in acute, rather than chronic, hepatitis C [198,199].

It has been proposed that intrahepatic T-lymphocytes expressing Fas ligand interact with Fas-positive hepatocytes in HCV infection to induce hepatocellular apoptosis. Indeed, up-regulation of Fas expression on hepatocytes as well as induction of Fas ligand in T-lymphocytes have been found to correlate with severity both pro- and anti-apoptotic effects depending on cell type, experimental conditions or other inadvertent factors [186–196].
of liver disease [190–204] and amount of hepatocyte apoptosis [205]. Patients with chronic hepatitis C have significantly higher Fas serum levels than healthy controls [206], and Fas prevalence is markedly higher in HCV-infected than in HCV-negative hepatocytes [207]. Finally, liver cell apoptosis in chronic hepatitis C had a positive correlation with the grading of histological activity and the amount of infiltrating CD8+ cells, but was not correlated with aminotransferase levels, HCV loads or genotypes [207]. Finally, measuring activity of caspases in sera of patients with chronic hepatitis C revealed a correlation with fibrotic liver injury [208]. Importantly, activation of caspase 3, a surrogate marker of apoptosis induction, can be observed in 7–20% hepatocytes [209], whereas the number of HCV-infected hepatocytes is estimated to be much lower [210]. Thus damage to uninfected bystander hepatocytes must make a major contribution to liver damage in chronic hepatitis C. Consequently caspase inhibitors, which are currently under clinical study in phase II trials, may become a novel tool to limit tissue damage in chronic hepatitis C [211].

TRAIL (TNF-related apoptosis-inducing ligand) is another member of the death factor family, which may have evolved in response to virus infection [212], and up-regulation of TRAIL receptors and increased TRAIL sensitivity are induced upon virus infections [213]. Moreover, type 1 and 2 IFNs induce expression of TRAIL and its receptor [212,214]. In chronic hepatitis C, TRAIL may be involved in the induction of hepatocyte steatosis [215]. Nevertheless, the role of the TRAIL/TRAIL receptor system remains poorly understood in chronic hepatitis C in particular, because viral proteins can both inhibit and enhance TRAIL-mediated pathways of apoptosis [216,217].

CONCLUSIONS

In HCV infection, a variety of different mechanisms mediated by the host’s immune system may play a role in the elimination of infected cells and inflammatory liver damage (Figure 1). Studies in viral hepatitis have largely been focused on measuring antigen-specific immune responses. HCV-specific cells can now be studied in peripheral blood and the liver without prior manipulation by isolation procedures and tissue culture conditions owing to novel sensitive techniques, such as the use of peptide tetramer complexes in flow cytometry. Although we can use these tools to investigate how the immune system controls HCV infection, the reasons why immunity frequently fails to successfully eliminate HCV still remain unclear. Our understanding of humoral immunity, in particular the role of neutralizing antibodies, is still incomplete, and we also do not know whether the test systems used to study cellular immunity provide adequate information on surrogate markers of protective cellular immunity. Certainly, CD4+ and virus-specific CD8+ T-cells are involved in the clearance of HCV-infected cells, but may exert significant liver damage.

On the other hand, the available data indicate that HCV exploits multiple and complex strategies to subvert the host’s innate and adaptive immune responses. In an attempt to defend against HCV infection, the immune system recruits and activates a variety of different HCV-specific as well as non-specific effector mechanisms. If these processes work correctly, they enable successful elimination of virus-infected cells and are beneficial for the host. However, de-regulation of cytotoxic cells and continued activation of apoptosis pathways will result in persistent liver damage, scarring and, ultimately, progression to cirrhosis. The outcome of HCV immune activation, viral clearance compared with chronic inflammation, is probably determined during the first few months of infection and, in this phase, several issues concerning the relative contributions from elements of innate and adaptive immunity, such as NK cells, NK T-cells and γδ T-cells as well as conventional CD4+ and CD8+ T-cells, remain to be resolved. Finally, we must still learn how to modify quality, magnitude, breadth and duration of antigen-specific immune responses in order to establish an effective immunotherapy against chronic hepatitis C.

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