NATURE|Vol 436|18 August 2005|doi:10.1038/nature04079

Adaptive immune responses in acute and chronic hepatitis C virus infection

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The hepatitis C virus (HCV) persists in the majority of infected individuals and is a significant cause of human illness and death globally. Recent studies have yielded important insights into immunity to HCV, in particular revealing the central role of T cells in viral control and clearance. Other key features of adaptive immune responses remain obscure, including mechanisms by which T cells control HCV replication, the role of antibodies in conferring protection and how cellular and humoral immunity are subverted in persistent infection.

The hepatitis C virus is among the most successful of all persistent human viruses. With a compact RNA genome thought to encode only 11 proteins, HCV persists in up to 70% of those infected by successfully undermining virus-specific immunity while leaving host defences to other infectious agents intact. An estimated 170 million individuals are infected worldwide, and approximately 38,000 new infections occur annually in the United States alone[1]. Twenty percent of persistently infected individuals will develop liver cirrhosis, and hepatocellular carcinoma occurs in up to 2.5% (ref. 1). There is as yet no vaccine against HCV. Furthermore, current anti-viral therapy is expensive, relatively toxic and effective in only 50–60 % of patients treated[2]. Understanding adaptive immunity to this virus is crucial for the design of effective strategies to control HCV both in the infected individual and globally.

The outcome of HCV infection is determined within six months of exposure to the virus. Acute infection is often unrecognized because symptoms are usually mild or absent. Initial views of immunity to HCV were therefore largely shaped by studies of chronically infected individuals (reviewed in ref. 3). Recent prospective studies of humans at high risk of HCV exposure and of experimentally infected chimpanzees have provided a more complete picture of adaptive immunity to the virus. In particular, control of acute primary viral replication is associated with expansion of antiviral $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T cells. Moreover, immunological memory conferred by spontaneous resolution of acute hepatitis C does not protect against reinfection, but does substantially reduce the risk of persistence upon re-exposure. Here we outline features of successful adaptive immune responses to HCV and review current concepts of evasion strategies that might explain defects in humoral and cellular immunity in those individuals who develop persistent infections.

The humoral response to hepatitis C virus infection

Virus-specific antibodies are usually detectable approximately 7–8 weeks after HCV infection[4]. Whether antibodies neutralize HCV infectivity is still incompletely understood. In support of a protective role, HCV infectivity for chimpanzees has been neutralized by *in vitro* treatment with antibodies[5], and infection outcome in humans was predicted by sequence changes in the hypervariable-1 region of the E2 envelope glycoprotein, a major target of the antibody response, that occurred simultaneously with antibody seroconversion[6]. These sequence changes are thought to represent escape mutations, and so HCV almost certainly adapts to immune selection pressure exerted by antibodies and, as will be discussed below, $CD8^+$ T lymphocytes. On the other hand, the role of naturally acquired antibodies in protection has been questioned because they do not prevent reinfection of immune chimpanzees or humans[7,8]. Moreover, resolution of HCV infection can occur without the development of anti-HCV antibodies in chimpanzees[9] and in the absence of seroconversion by standard assays in humans[10].

Delineating the contribution of anti-HCV antibodies in infection outcome will probably depend on development of *in vitro* culture models for measuring their neutralizing capacity. Important progress towards this goal has recently been described. Infectious lentiviral pseudotype particles bearing native HCV envelope glycoproteins have been used to show cross-viral genotype neutralization of HCV by serum antibodies from chronically infected subjects[11–13]. However, these antibodies are rare in individuals who resolve infection[11–13], suggesting that other mechanisms of adaptive immunity are important contributors to viral clearance.

T-cell immunity, HCV replication and liver damage

Acute infection

HCV genomes and proteins have been visualized within human and chimpanzee hepatocytes[14,15], but estimates of the proportion infected are variable and uncertain. Viraemia, or presence of HCV RNA genomes in the bloodstream, is therefore used as a surrogate for intrahepatic HCV replication. Three broad patterns of acute phase replication were identified shortly after the discovery of HCV[16] and are now being reinterpreted as T-cell responses to viral proteins are defined. HCV RNA genomes appear in the plasma within a few days of infection and typically peak 6–10 weeks later regardless of outcome[16–18]. A pattern of poorly controlled viraemia predicts persistence (Fig. 1a) that may be explained, at least in part, by the failure of some individuals to generate detectable $CD4^+$ and $CD8^+$ T-cell responses[9,19–22] (Fig. 1a). Further along the spectrum are individuals with transient (Fig. 1b) or permanent (Fig. 1c) control of viral replication that is temporally associated with late onset HCV-specific $CD4^+$ and $CD8^+$ T-cell responses[9,19–22]. Why cellular immunity is often delayed for several weeks, even in humans and chimpanzees who ultimately clear the infection, is not yet understood[9,22–24]. Viraemia also often rebounds after the infection is substantially controlled[21–23] and can be a prelude to viral persistence.

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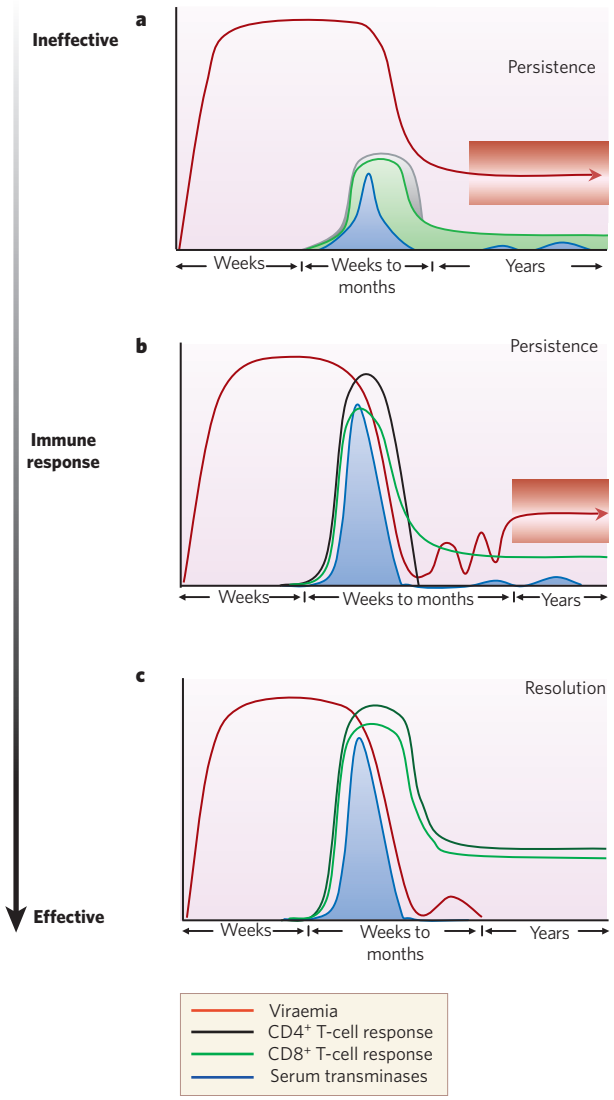

Figure 1 | Schematic representation of the cellular immune response during acute HCV infection. **a**, Viraemia (red line) is present early, and although it usually falls from peak levels, it is never controlled. Persistent infection ensues, with plasma viral levels that vary widely between individuals. $CD4^+$ and $CD8^+$ T-cell responses (black and green lines, respectively) and rises in serum transaminase (blue) in this setting remain poorly characterized, and may be weak or even absent. Progressive shading indicates the high degree of variability in responses between individuals. **b**, Viraemia may persist for many weeks in the absence of a demonstrable cellular immune response. The delayed onset of $CD4^+$ and $CD8^+$ T-cell responses is associated with transient control of viraemia and a variable rise in transaminases. However, following contraction of the $CD4^+$ T-cell response, viraemia rebounds and ultimately infection persists. Detectable $CD8^+$ T-cell responses may persist despite chronic viraemia. Shading indicates variability in viral load between individuals in the chronic phase. **c**, Although viraemia arises early and T-cell responses are delayed, the virus becomes undetectable in plasma following emergence of $CD4^+$ and $CD8^+$ T-cell responses, which are often coincident with a variable rise in serum transaminases. A rebound in viraemia may occur before final viral clearance.

Liver damage is monitored by levels of hepatic enzymes (transaminases) released into the serum following hepatocellular injury. Acute hepatitis C is probably immunopathological because it coincides temporally with expansion of virus-specific $CD8^+$ T cells or cytotoxic T lymphocytes (CTLs)[9,22–24] and their acquisition of an activated phenotype[25]. Curiously, acute infection can sometimes resolve without a serum transaminase increase. This could reflect non-cytolytic control of some infections by T-cell-derived cytokines such as interferon-γ (IFN-γ)[23,26,27], but it is also possible that relatively few hepatocytes are infected in these individuals, which would limit the extent of CTL-mediated liver damage.

Chronic infection

As discussed in detail below, HCV-specific $CD8^+$ T cells can survive for years in the persistently infected liver and thus might at least partially control ongoing viral replication and/or contribute to progressive liver disease. With regard to viral replication, it is noteworthy that levels of viraemia remain relatively stable over time in subjects with chronic infections even though they can differ widely between individuals (Fig. 1). Data regarding the relationship between intrahepatic CTLs and viral load have been conflicting[28–31], and thus whether they contribute to control of HCV replication in chronic hepatitis C or explain the wide variation in viraemia between individuals is not known. Similarly, although virus-specific CTLs are concentrated within the liver in chronic infection[29,30,32], they have only inconsistently been correlated with disease severity[29,31]. Some studies have documented an association between elevated transaminases and liver infiltration by $CD8^+$ T cells[31,33], but it is possible that only a minority of the infiltrate is HCV specific[34]. Intrahepatic pro-inflammatory cytokine messenger RNA (mRNA) levels have been correlated with severity of portal inflammation and liver fibrosis[35,36]. However, it is unclear whether HCV-specific liver infiltrating lymphocytes are the major cellular source of pro-inflammatory cytokines, such as IFN-γ, in HCV-associated chronic hepatitis[36]. Importantly, recruitment of other inflammatory cell types such as macrophages that can mediate tissue injury also occurs in chronic hepatitis C[36]. Thus, studies to weigh the contribution of both antigen-specific and antigen-independent mechanisms in chronic hepatocellular injury are needed.

Parsing T-cell responses in resolving and persistent infections

Features that distinguish T-cell responses providing transient versus permanent viral control are not fully defined. Comparisons have focused on the number of epitopes targeted by acute-phase T cells, their frequency in blood and their fate as infections clear or persist. $CD8^+$ T-cell responses are perhaps the best characterized. Successful responses generally target multiple major histocompatibility complex (MHC) class I-restricted epitopes in structural and non-structural HCV proteins[9,24,37,38], and frequencies against individual epitopes often exceed 3–4% (ref. 37). Interestingly, late expansion of HCV-specific $CD8^+$ T cells may be accompanied by a further delay in their production of IFN-γ[22,24,37], although the significance of this phenomenon to infection outcome is uncertain.

Infections that follow a chronic course are usually marked by low frequencies of CTLs targeting few epitopes[28,39–42], although occasionally the strength and breadth of the response can approximate those of resolving infections[43]. HCV-specific CTLs are not necessarily deleted as infections persist and, although difficult to detect in blood, can survive in the persistently infected liver for years[29,30,32,44,45] and in some cases target a surprisingly broad array of epitopes. For example, intrahepatic CTL populations recognizing eight HCV epitopes were consistently recovered from a chimpanzee throughout years of persistent infection[45,46], and CTL clones isolated at diverse timepoints often retained use of identically recombined T-cell receptor (TCR) α and β chains[47], providing genetic evidence of their remarkable stability despite ongoing viral replication. These intrahepatic populations are almost certainly a remnant of a once robust acute phase response.

Strong $CD8^+$ T-cell immunity in acute resolving hepatitis C is matched by vigorous, sustained $CD4^+$ T-cell proliferation to multiple recombinant structural and non-structural viral proteins[19–21]. By contrast, HCV antigen-driven proliferation in individuals who develop persistent infections is usually weak or absent when compared with spontaneously resolving infections[19–22]. Nevertheless, transient $CD4^+$ T-cell proliferative responses that are indistinguishable from those in acute resolving infections have been described[21,22], and although possibly rare do indicate that HCV can evade what appears to be initially

robust $CD4^+$ T helper (T_H) cell activity. Very importantly, permanent loss of this HCV-specific proliferation during acute hepatitis C predicts persistence[20,21].

In addition to blunted and transient proliferative responses, $CD4^+$ T cells from persistently infected individuals target few MHC class II-restricted epitopes. For instance, in one study very few HCV-specific $CD4^+$ T cell lines were established from humans with persistent infection, whereas those with resolved infections recognized up to 14 different HCV epitopes[48]. Despite the broad diversity of epitopes targeted, preliminary studies in a chimpanzee with acutely resolving infection suggest that evolution of a multi-specific $CD4^+$ T-cell response can be complex, initially focusing on a limited number of dominant epitopes and then spreading to additional targets only after viraemia is mostly controlled[49]. Potential consequences for infection outcome of a T_H response that is transiently narrow during a critical period of viral control have not yet been fully explored.

Memory T cells and HCV resolution

Control of viraemia is associated with the contraction of detectable virus-specific $CD4^+$ and $CD8^+$ T-cell responses, almost certainly by programmed death as normally occurs at the end of cellular immune responses. This late phase of the response is often marked by resurgent HCV replication that may reflect a delicate balance between slow clearance of intrahepatic viral genomes and an easing of immune control as virus-specific T cells die (Fig. 1b, c). As noted above, this rebound in viraemia can lead to viral persistence after a transient period of control, and it may reflect active evasion of T-cell responses in the very late stages of acute hepatitis C. In contrast, infections that are successfully controlled result in durable memory populations. For instance, most subjects who resolved an accidental infection with a single source of HCV-contaminated immunoglobulin[40] had strong HCV-specific T-cell immunity in blood 18 years later, even though serologic responses to the virus had waned in the majority. Durable intrahepatic memory is probably also established, because T cells recognizing HCV antigens have been recovered from the livers of chimpanzees several years after spontaneous clearance of infection[24].

T-cell memory may explain a substantially lower rate of HCV persistence in re-exposed humans with a history of acute resolving hepatitis C[50]. In support of this concept, immune chimpanzees have been shown to be susceptible to reinfection, but there were marked reductions in the duration and peak of viraemia[24,51,52] coinciding temporally with massive $CD4^+$ and $CD8^+$ T-cell recall responses[24] (illustrated schematically in Fig. 2). Importantly, antibody-mediated depletion of $CD8^+$ T cells from immune chimpanzees prolonged viraemia after rechallenge with the same HCV strain, and viral clearance was precisely correlated with recovery of HCV-specific $CD8^+$ T cells within the liver[24]. Treatment with anti-CD4 antibodies in a parallel experiment resulted in HCV persistence, revealing the importance of memory $CD4^+$ T cells to infection outcome[38] (Fig. 2).

Mechanisms of HCV persistence

Descriptions of the repertoire, frequency and fate of $CD4^+$ and $CD8^+$ T cells in acute and chronic hepatitis C have provided limited insight into their subversion by HCV. A variety of postulated mechanisms are summarized in Fig. 3 and discussed below. It should be noted, however, that few enjoy substantial experimental support, and none can fully account for the inability to generate or sustain a $CD4^+$ T_H cell response.

It is important to emphasize that, in the absence of advanced liver disease, defects in cellular immunity appear exquisitely HCV specific. Any proposed model of evasion should therefore explain an antigen-specific lesion in cellular immunity lasting for decades. This would seem to exclude, for example, direct cytopathic destruction of infected T lymphocytes or antigen-presenting cells (APCs). Indirect suppression of T-cell activity by viral proteins binding ubiquitous surface receptors is also not easily reconciled with apparently normal immune function in persistently infected individuals. Immunomodulatory activity of HCV proteins like the viral nucleocapsid is nonetheless supported by *in vitro* cell-culture models[53,54], and it is possible that threshold protein concentrations required for suppression are reached only in the infected liver or perhaps during acute hepatitis C when viral replication peaks.

A number of mechanisms consistent with an HCV-specific defect in immunity have been proposed. Two that are conceptually attractive despite limited experimental support include an inability of effector T cells to move to the infected liver[23] and impaired antigen presentation. The potential for impaired antigen presentation in HCV infection merits attention because it could explain defects in immunity ranging from an apparent absence of HCV-specific T cells in some individuals to substantially delayed or nonsustained responses in others. Dendritic cells (DCs), the primary dedicated APC, may be particularly susceptible to a potentially potent and multi-faceted attack on innate immune mechanisms (see the review in this issue by Gale and Foy, page 939) essential for their maturation and effective antigen presentation to T cells. For instance, although natural killer (NK) cells are an important direct mediator of the innate immune response that have been implicated in the control of HCV infection[55] and may be inhibited by the HCV envelope E2 protein[56,57], they are also potent activators of DCs. However, recent data from an *in vitro* cell-culture model indicate that negative regulatory signals delivered to NK cells in the persistently HCV-infected liver could interrupt this ability to mediate DC maturation[58]. Inhibition of antigen presentation in HCV infection might also be mediated more directly by the effects of viral proteins on DCs. Monocyte-derived DC from the blood of persistently infected individuals may display defects in maturation or stimulation of allogeneic T-cell responses[59–61]. These have not been consistent findings in the literature, however[62,63], and permanent global defects in DC function extending into the chronic phase of infection are not clinically evident. On the other hand, a transient defect in DC function during acute hepatitis C could be sufficient to alter the timing or vigour of T-cell immunity and favour persistence.

Three other mechanisms of immune evasion that include mutational escape of epitopes, functional anergy and regulatory T-cell activity enjoy more substantial experimental support, even if their role in HCV persistence is not proven. Most of the published studies so far have focused on how these mechanisms alter HCV-specific $CD8^+$ T cell immunity; their relevance to loss of $CD4^+$ T cell help is still mostly unexplored.

Mutational escape of HCV epitopes

In common with other highly mutable RNA viruses, HCV is well adapted to generate genomic diversity that can be exploited to evade $CD8^+$ T cells. The viral NS5b protein, an error-prone RNA-dependent RNA polymerase, could be considered an immune evasion molecule because it prolifically generates minor viral variants[64] with the potential to evade recognition. Epitopes may be lost because amino-acid substitutions result in proteasomal destruction or impaired binding to MHC molecules[65]. Alternatively, amino-acid changes can alter CTL recognition of variant peptide—MHC complexes[65].

One key criticism of the mutational escape hypothesis is that, even with a high mutation rate, HCV is unlikely to evade multi-specific CTL responses found in some individuals who develop chronic infection. Delaying expansion and acquisition of effector functions by $CD8^+$ T cells for weeks during acute infection could favour accumulation of variants with the potential to evade immune control. This delay on its own is probably not sufficient to facilitate escape from a multi-specific CTL response. When combined with the spontaneous loss of $CD4^+$ T-cell help that precedes HCV persistence, however, $CD8^+$ T-cell function might be impaired to the point where they are no longer capable of mediating viral clearance, but still exert sufficient immune pressure to select for variant viruses. The concept that lack of help undermines the effectiveness of HCV-specific $CD8^+$ T cells is supported by recent data from immune chimpanzees[38], where re-challenge with the same HCV strain following antibody-mediated depletion of $CD4^+$ T cells resulted in persistent infections associated with CTL escape mutations in multiple MHC class I-restricted epitopes[38] (Fig. 2). Other immuno-

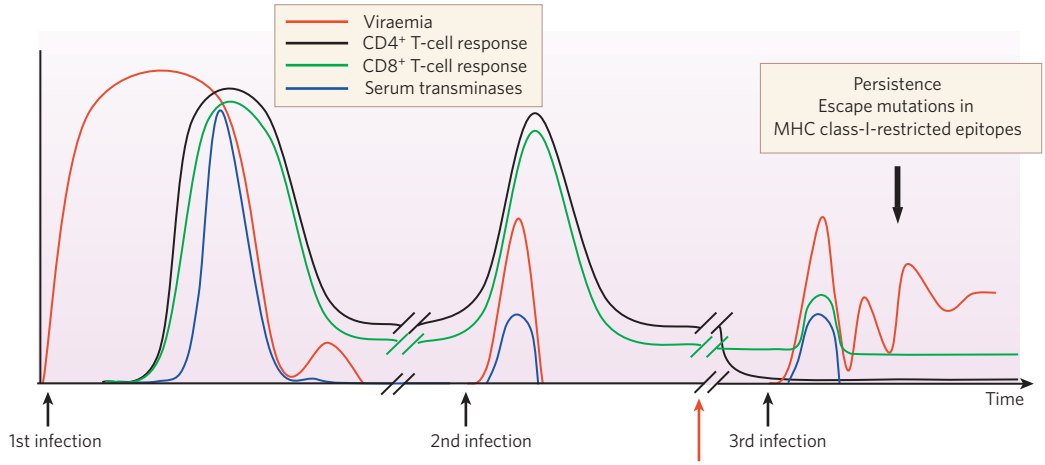

Figure 2 | Schematic representation of memory response to homologous HCV re-challenge, and outcome of a third challenge following CD4+ T-cell depletion. Following initial rechallenge, the onset of viraemia (red line) is associated with relatively rapid onset of strong $CD4^+$ and $CD8^+$ memory T-cell responses (black and green lines, respectively). The level of viraemia is lower than in primary infection, and viral clearance is more rapid. Associated transaminase rises (blue line) are also reduced. Following $CD4^+$ T-cell depletion (indicated by a red arrow), viral rechallenge is associated with a weaker $CD8^+$ T-cell response, which partly controls viraemia but is followed by viral rebound and persistence as escape mutations emerge in MHC class-I-restricted epitopes.

logical factors such as the TCR diversity used in HCV-specific $CD8^+$ T-cell responses may also be a factor in the development of escape mutations[47], with narrower responses less able to constrain the emergence of viral variants.

$CD8^+$ T-cell mediated selection pressure against HCV was first demonstrated in the chimpanzee model[45]. Evidence for escape mutations in human HCV infections has accumulated slowly, especially as the sequence of the transmitted virus is rarely known. Early indirect evidence for CTL escape mutations was inferred by comparison of circulating sequences in chronically HCV-infected individuals with prototypical epitopes[66]. These data have recently been reinforced by studies where viral sequences were available either from the source or during early infection, in which the development of persistent viraemia was associated with escape mutations in targeted MHC class-I-restricted epitopes[67–69]. Recent population-based approaches have also provided further support for CTL-driven HCV evolution[67,70].

Studies in human (HIV) and simian (SIV) immunodeficiency virus infections indicate that escape mutations may exact and be constrained by a 'fitness cost' to replication that varies between epitopes. Following transmission of HIV or SIV to hosts expressing non-selecting MHC, certain epitopes with potentially high associated fitness cost revert to wild-type sequence[71,72]. Others, presumably associated with low replicative impairment, revert slowly or not at all[71,73]. Recent analyses of HCV-infected cohorts have demonstrated that mutations tending toward viral consensus do occur in prototypical epitopes unrestricted by host MHC alleles[67,69,70], which may indicate reversion of virus to more replicatively fit ancestral sequences. However, further studies are required to define the extent to which fitness cost constrains the development of escape mutations within HCV epitopes.

It is important to note that the phenomenon of CTL escape mutation may influence not just viral quasispecies within individuals, but also the dominant viral species circulating in human populations. Escape mutations within HIV epitopes restricted by common MHC class I alleles may impart a 'footprint' of associated polymorphisms upon viral species within a population[74]. Furthermore, CTL escape mutations in epitopes with negligible fitness cost may persist in HIV genomes transmitted to individuals without the restricting MHC class I allele, to the point of becoming the dominant sequence observed at a population level[73]. Thus, immunodominant HIV epitopes restricted by MHC alleles that are common within a population may be lost from circulating viral species[75]. Preliminary evidence indicates that commonly expressed HLA alleles may also influence HCV sequences at a population level (S. Gaudieri, personal communication).

The relationship between fitness cost associated with escape mutation and clearance of HCV infection remains largely unexplored. It is conceivable that $CD8^+$ T cells mediating viral clearance target a set of protective epitopes functionally constrained from mutation. However, there is as yet no experimental support for this hypothesis in an infection where patterns of epitope dominance are usually neither obvious nor easily predicted from HLA class I haplotypes of infected subjects[76].

The hypothesis that $CD4^+$ T cells can also exert immune selection pressure is attractive because, as noted above, the response might initially focus on a limited set of dominant epitopes and may be influenced by MHC class II allelic associations with infection outcome[3]. However, although amino-acid substitutions in HLA class II epitopes of HCV can skew patterns of cytokine expression[77] and antagonize[78] or abrogate[79] T_H cell activity, few MHC class II epitopes have been studied so far and despite persistent HCV replication, amino-acid substitutions are rarely observed[80]. Perhaps most importantly, formal statistical proof that the rate of mutations that result in amino-acid substitutions is increased in MHC class II-restricted versus unrestricted epitopes or flanking regions of the HCV genome is still lacking.

It should be noted that many T-cell epitopes are intact in persistently replicating HCV genomes and do not mutate despite an intense and focal $CD8^+$ T-cell response[43]. These observations suggest that mutational escape is not the only mechanism for evading antigen-specific T cells. Experimental evidence for two additional mechanisms, functional anergy and generation of regulatory T-cell populations, indicate that there are multiple complementary pathways to establish or maintain viral persistence in the presence of HCV-specific T cells.

Deletion or anergy

Although deletion of antigen-specific $CD8^+$ T cells has been demonstrated in chronic murine lymphocytic choriomengitis virus (LCMV) infection[81], no data are yet available as to whether such mechanisms affect HCV-specific $CD8^+$ T cells. However, recent studies of HCV-specific $CD8^+$ T cells have indicated that these cells may be functionally impaired, or anergic, in chronic disease[41,82,83] and, consistent with this loss of function, may exhibit phenotypic alterations characteristic of early stages of differentiation[84]. Interestingly, congruent with chronic HCV infection, in LCMV infection $CD8^+$ T-cell anergy has been associated with failing or absent virus-specific $CD4^+$ T-cell responses[85,86].

Although these findings suggest a possible role for $CD8^+$ T-cell anergy in HCV persistence, their significance remains uncertain as virus-specific $CD8^+$ T cells were also impaired after resolution of infection in at least one of these studies[82]. Furthermore, phenotypical alterations of CMV-specific $CD8^+$ T cells have also been described in chronic HCV infection despite a lack of clinically evident dysfunction[87]. Moreover, the majority of phenotypical studies of HCV-specific

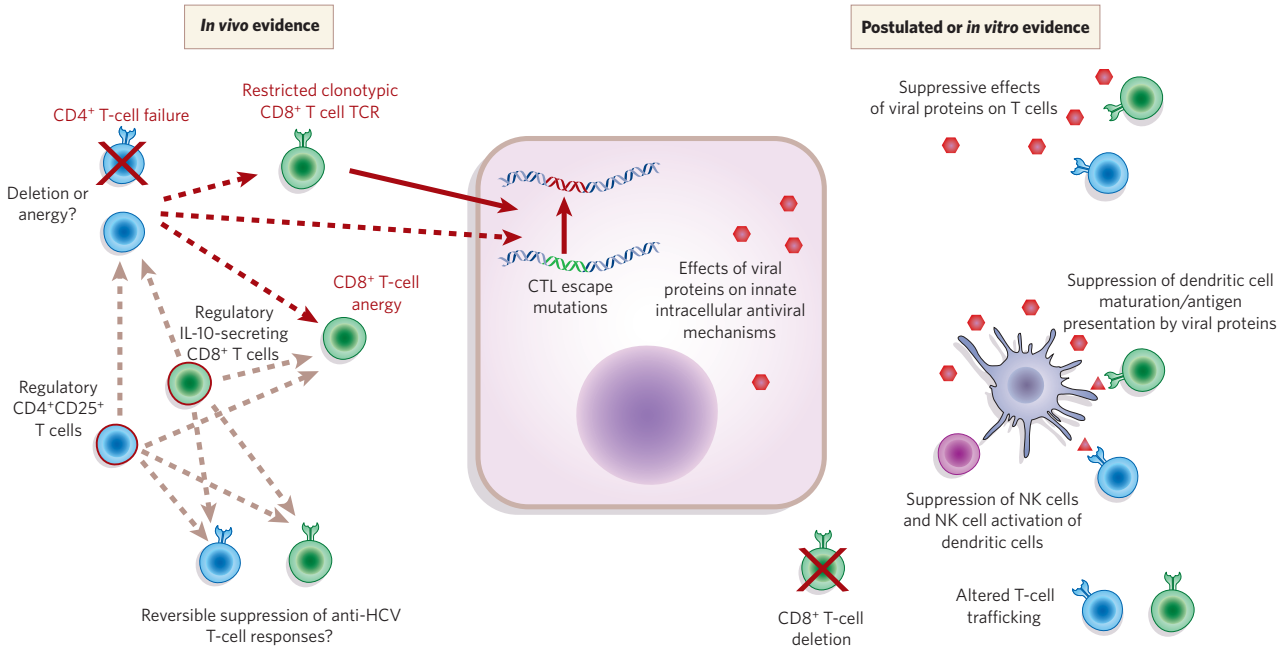

Figure 3 | Possible mechanisms of immune evasion by the hepatitis C virus. Red lines and headings represent mechanisms for which there is the most supporting *in vivo* experimental data; dotted lines indicate that although the mechanisms indicated may be involved in viral persistence, their pathways remain unclear. Large crosses indicate deletion of cells, whereas triangles indicate inhibition of antigen presentation. Interactions of viral products with intracellular pathways and the consequent effects on innate immunity are covered in the accompanying review by Gale and Foy (page 939).

$CD8^+$ T cells have been performed on peripheral blood; it remains possible that the characteristics of virus-specific CTLs may differ within the liver, the primary site of infection. Thus, further examination of HCV-specific T cell phenotype and function is required before definitive conclusions can be drawn.

Although persisting CTL responses are detectable in some chronically HCV-infected individuals, virus-specific $CD4^+$ T cell responses are generally weak or absent when assessed using functional methods of identification[20,40,48,88]. However, it remains unclear whether HCV-specific $CD4^+$ T cells are present but functionally impaired or have been lost from the repertoire. In a recent study employing MHC class II tetramers to identify antigen-specific cells independent of function, HCV-specific $CD4^+$ T cells were not detected in chronically viraemic individuals[89]. By contrast, a study in which upregulation of the activation marker CD25 in response to antigenic stimulation was used to identify HCV-specific $CD4^+$ T cells indicated that such cells might be present, but functionally altered[90]. It is also possible that loss of virus-specific $CD4^+$ T cells may be somewhat epitope dependent, with cells specific for the core antigen being more readily detected that those directed against the non-structural proteins[91,92]. Interestingly, a sub-population of $CD4^+$ T cells specific for HCV core that persist in chronic infection may be characterized by altered patterns of cytokine production including secretion of the anti-inflammatory cytokine interleukin (IL)-10 (ref. 91) or impaired secretion of the survival cytokine IL-2 (ref. 92).

Regulatory T-cell populations

A subset of HCV-specific $CD8^+$ T-cell lines derived from the liver of a persistently infected subject was found to produce the immune suppressive cytokine IL-10, providing the first suggestion of MHC class-I-restricted antigen-specific regulatory activity with the potential to suppress antiviral T cells[93]. These data were reinforced by more recent observations that intrahepatic $CD8^+$ T cells from persistently infected subjects suppressed the *in vitro* proliferative responses of liver-derived lymphocytes in an HCV-specific and IL-10-dependent manner[94].

In addition to the presence of possible intrahepatic regulatory $CD8^+$ T-cell populations, the proportion of $CD4^+CD25^+$ regulatory T cells may be elevated in the peripheral circulation of some chronically HCV-infected subjects when compared with recovered or uninfected individuals[95–97]. Depletion of $CD4^+CD25^+$ T cells from peripheral blood increased the frequency of functional HCV-specific $CD8^+$ T cells in *in vitro* assays[95–98], indicating the potential for regulatory activity to suppress antiviral responses. Interpretation of this observation is not straightforward, however, because suppression extended to $CD8^+$ T cells targeting cytomegalovirus, Epstein–Barr virus and influenza virus[97,98]. Our understanding of regulatory $CD4^+$ T cells in viral persistence is further complicated by uncertainty over their localization to secondary lymphoid organs or liver where antigen-specific interactions relevant to HCV persistence are more likely to occur.

In summary, HCV persistence is predicted by a failure to generate or sustain $CD4^+$ T-cell responses, and this outcome can be recapitulated by anti-CD4 antibody treatment of immune chimpanzees, suggesting that an HCV-specific loss of T-cell help is a central event required for immune evasion. Although lacking in critical detail and experimental proof, one working model of HCV persistence is that the virus sets in train a series of cascading events where interference with innate immunity causes a defect in $CD4^+$ T-cell help. Depending on the severity of this impairment, $CD8^+$ T cells essential for viral clearance either succeed or fail. Absence of adequate help could provide a common explanation for two of the major defects observed in the $CD8^+$ T-cell compartment, specifically mutational escape of MHC class-I-restricted epitopes and functional anergy. It is, however, too soon to discount the importance of other more direct mechanisms of $CD8^+$ T cell inactivation, particularly in an organ like the liver with its own unique populations of APCs that can modulate immunity[99].

Vaccines for HCV: opportunities and risks

The temporal kinetic relationship between the onset of T-cell responses and control of viraemia, and the altered outcome of infection in immune chimpanzees depleted of $CD4^+$ and $CD8^+$ subsets, clearly indicate that cellular immunity is critical in prevention of HCV persistence. These findings, combined with the observation that immunity can even provide protection against different HCV genotypes[100], have raised hopes for a safe and effective vaccine (see the review in this issue by Houghton and Abrignani, page 961). Despite

these positive developments, from the point of view of adaptive immunity to the virus, it may be too soon to predict success. Our understanding of humoral immunity to HCV, particularly the potential for antibody cross-reactivity among genetically diverse isolates and the role of neutralizing antibodies, is still rudimentary. With regard to cellular immunity, we still lack knowledge of how HCV is cleared and it is not at all certain that robust virus-specific responses as assessed by currently employed assays, in particular production of IFN-γ, are adequate surrogates for protective T-cell immunity. Perhaps the greatest risk comes from our poor insight into how HCV inactivates primed $CD4^+$ T_H cells even after they have contributed to transient control of viraemia. Our ability to safely harness protective immunity by vaccination may depend on the answers to these very basic questions. ■

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Acknowledgements Our research was supported by Public Health Service grants to C.M.W. D.G.B. was supported by a C.J. Martin Fellowship from the National Health and Medical Research Council of Australia and an AstraZeneca Fellowship in Medical Research from the Royal Australasian College of Physicians.

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