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TOPIC HIGHLIGHT

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CD4+ T cell responses in hepatitis C virus infection

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Abstract

Hepatitis C virus (HCV) infection is a major cause of liver damage, with virus-induced end-stage disease such as liver cirrhosis and hepatocellular carcinoma resulting in a high rate of morbidity and mortality worldwide. Evidence that CD4+ T cell responses to HCV play an important role in the outcome of acute infection has been shown in several studies. However, the mechanisms behind viral persistence and the failure of CD4+ T cell responses to contain virus are poorly understood. During chronic HCV infection, HCV-specific CD4+ T cell responses are relatively weak or absent whereas in resolved infection these responses are vigorous and multispecific. Persons with a T-helper type I profile, which promotes cellular effector mechanisms are thought to be more likely to experience viral clearance, but the overall role of these cells in the immunopathogenesis of chronic liver disease is not known. To define this, much more data is required on the function and specificity of virus-specific CD4+ T cells, especially in the early phases of acute disease and in the liver during chronic infection. The role and possible mechanisms of action of CD4+ T cell responses in determining the outcome of acute and chronic HCV infection will be discussed in this review.

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Key words: Hepatitis C virus; CD4 T cells; HLA class Ⅱ; Immune responses; Cytokines; Interleukin 2; Proliferation; Escape; Exhaustion

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major cause of liver damage, with virus-induced end-stage disease such as liver cirrhosis and hepatocellular carcinoma resulting in a high rate of morbidity and mortality worldwide. Since the discovery of the virus, considerable evidence has emerged that CD4+ T cell responses to HCV play a key role in the outcome of infection. However many questions remain and these will be discussed in this review.

Cellular immune responses, involving both CD8+ cytotoxic T lymphocytes (CTL) and CD4+ T-helper cells, play an essential role in the control of HCV infection, as they do in other persistent viral diseases. Whereas CTLs are traditionally thought to be the main effector cells that eliminate HCV-infected cells[1], it is clear that HCV-specific CD4+ T cells also play a critical role. These cells can potentially act in multiple ways and are central to the initiation and maintenance of adaptive immunity. Two likely major roles are in providing help for CD8+ T cells by cytokine production and activation of antigen-presenting cells, but there are multiple other mechanisms of action including direct antiviral effects, a role in B cell maturation, and regulatory functions.

A very clear example of the overall importance of HCV-specific CD4+ T cell responses *in vivo* is an experiment where the antibody-mediated depletion of CD4+ T cells before re-infection of two immune chimpanzees was performed. Such depletion resulted in persistent, low-level viraemia despite functional intra-hepatic memory CD8+ T cell responses[2]. In this experiment incomplete control of HCV replication by memory CD8+ T cells in the absence of adequate CD4+ T cell help was associated with emergence of viral escape mutations in Class I MHC-restricted epitopes and failure to resolve HCV infection[2]. This experiment is important in that it shows that CD4+ T cells are necessary for resolution of HCV infection. However, in this case the exact function of the CD4+ T cells was not elucidated. The fact that CD8+ T cell responses were maintained and were functional (inducing viral escape) suggests that their role is not purely in providing support for CD8+ T cell responses.

Overall, most data on CD4+ T cells comes from experiments in infected persons, particularly those

comparing chronic and resolved infection. During chronic HCV infection, HCV-specific CD4+ T cell responses are typically described as weak or absent whereas in resolved infection these responses are generally vigorous and multispecific. However, much of the data is derived from cross sectional studies and also based on analysis of one function-proliferation *in vitro*. There is no doubt that CD4+ T cell responses in resolved infection and in persistent infection look very different, but how they diverge and what the true functionality of the populations are is not yet clear. In our view, given their central importance in adaptive immunity, understanding why CD4+ T cell responses may fail in acute infection is the key question in HCV pathogenesis. Current data on the function and role of virus-specific CD4+ T-helper cells in acute and chronic HCV infection will be reviewed here and models discussed.

CD4+ T CELL RESPONSES IN ACUTE HCV INFECTION

Clearly, the best place to study the CD4+ T cell response against HCV is at the site of infection, but CD4+ T cell responses in the liver of acute HCV infection in humans have not been characterized to date. In chimpanzees, liver CD4+ T cells have been expanded with anti-CD3 and IL-2 and then tested for proliferation in response to HCV proteins[3]. In those who failed to control the virus, no CD4+ T cell responses were identified whereas in those chimps who transiently or permanently controlled the virus, strong proliferative CD4+ T cell responses were detectable. This experiment is limited by the need to expand the cells first *in vitro*, which might exclude certain cell populations, especially at the peak of infection. What is interesting is not only the dichotomy between those animals that cleared virus and those that did not, but also the "transient" group. This group does mount an early CD4+ response against HCV, but it is not sustained. The clinical phenomenon of transient control is common in human infection, with "yo-yo" patterns often described, and will be discussed further below.

All human studies in acute disease have been performed on blood. In these, overall, acute resolving HCV infection has been associated with a sustained response by HCV-specific CD4+ T cells. In one representative study patients who failed to clear the virus were divided into two groups[4]. Group 1 was unable to mount an HCV-specific CD4+ T cell response and developed chronic HCV. In Group 2, HCV RNA was cleared initially and was associated with strong HCV-specific CD4+ T cell responses. However, these responses diminished just before a rebound of viraemia that resulted in chronic infection. Therefore, a vigorous anti-viral CD4+ T cell response (as measured by proliferation assays) in the early and late phase of acute HCV seems necessary to achieve long-term viral control (CD4+/Th1 response). However, it is not sufficient (as in the chimpanzees), since those who mount such a response do not necessarily go on to clear virus.

Although what the Gerlach paper described is especially clear cut in relation to the "yo-yo" pattern, other groups have also reported that the vigour of the T cell response during the early stages of infection may be a critical determinant of disease resolution and control of infection[5]. That persistent infection can develop despite the presence of acute-phase HCV-specific CD4+ T cell responses has been shown in a study of healthcare workers exposed to needle-stick injuries[6]: although two individuals had strong HCV-specific CD4+ T cell proliferative responses in the acute phase with significant decreases in HCV RNA initially, they subsequently became chronically infected. This was an important study since the individuals were available for analysis shortly after infection-before clinical symptoms arose. Since responses are sometimes transient, it may be that in studies where patients are only analysed after they present clinically with acute infection, early T cell responses have been missed.

Such analyses have traditionally relied on proliferation. Using alternative techniques, it has been shown that evolution of the infection to chronicity can be associated with HCV-specific CD4+ T cells which survive initially despite failing to proliferate or produce IFN-γ: these diminish eventually as infection persists[7]. A study of a single patient using a novel approach with Class II MHC peptide complexes ("tetramers") revealed the loss of functionality of cells before the final loss of detection of such populations[8]. The failure of such responses in this patient occurred before the re-emergence of virus after transient control. This is important since it suggests that not all the failure of CD4+ T cell responses may occur as a consequence of prolonged viremia.

Overall, these studies suggest that a range of HCV-specific CD4+ T cell responses in the acute HCV phase can exist in blood and liver. In a subgroup of individuals, it may be that CD4+ T cell responses are not mounted initially, or are not detectable at the time of presentation. These individuals are very likely to develop chronic infection[9]. In another group, strong responses are detected and sustained, which is typically associated with resolution of infection. An important third group (probably the majority of patients) do mount a CD4+ T cell response initially, but loss of such responses is associated with progression to chronicity. What the differences are in these initial responses to HCV and why certain key CD4+ T cell responses are not sustained remain important questions in understanding HCV persistence. To understand this further it is necessary to consider the exact targets of the CD4+ T cell response.

TARGETS OF THE CD4+ T CELL RESPONSES IN HCV INFECTION

A more effective immune response against HCV could result from targeting more epitopes, mounting larger responses or targeting a key region. There is some evidence for all three. Overall, permanent resolution of infection has been related to both the breadth (number of CD4 epitopes targeted) and the magnitude of HCV-specific CD4+ T cell responses. While no one target has been identified as the key determinant, a number of candidates have been proposed.

Table 1 Displays the most commonly described CD4+ restricted epitopes

Amino acid position	HCV protein	Amino acid sequence	HLA-restriction	Reference
aa 21-40	Core	DVKFPGGGQIVGGVYLLPRR	DRB1*1101,DQB1*0301	Day, 2002
aa 31-45	Core	VGGVYLLPRR GPRLG	DRB1*1101	Godkin, 2001
aa 141-155	Core	GAPLGGAARA LAHGV	DRB1*1101	Godkin, 2001
aa 393-410	E2	GFATQRLTSLFALGPSQK	DRB1*1101	Frasca, 1999
aa 1241-1260	NS3	PAAYAAQGYKVLVLNPSVAA	DRB1*15, DRB1*0301 +	Day, 2002
aa 1248-1261	NS3	GYKVLVLNPSVAAT	DR4, DRB1*1101	Wertheimer, 2003
aa 1248-1267	NS3	GYKVLVLNPSVAATLGFGAY	DQB1*0301	Lamonaca, 1999
aa 1251-1259	NS3	VLVLNPSVA	DRB1*1101,DRB1*1201, DRB1*0401, DRB1*1302	Day, 2002
aa 1384-1401	NS3	VIKGGRHLIFCHSKKKCD	DRB1*15	Eckels. 1999
aa 1411-1426	NS3	GINAVAYYRGLDVSVI	DRB1*15	Gerlach, 2005
aa 1531-1550	NS3	TPAETTVRLRAYMNTPGLPV	DRB1*0701	Day, 2002
aa 1539-1554	NS3	LRAYMNTPGLPVCQDH	DRB1*15	Gerlach, 2005
aa 1581-1600	NS3	ENLPYLVAYQATVCARAQAP	DRB1*1001	Day, 2002
aa 1686-1705	NS4a	VVLSGKPAIIPDREVLYREF	DRB1*0301	Harcourt, 2003
aa 1746-1765	NS4b	IAPAVQTNWQKLETFWAKHM	DRB1*16 or DRB3*0202	Harcourt, 2003
aa 1767-1786	NS4b	NFISGIQYLAGLSTLPGNPA	DRB1*1104	Carlos, 2004
aa 1771-1790	NS4b	GIQYLAGLSTLPGNPAIASL	DRB1*0404	Day, 2002
aa 1806-1818	NS4b	TLLFNILGGWVAA	DRB1*0101	Gerlach, 2005
aa 1907-1926	NS4b	GPGEGAVOWMNRLIAFASRG	DRB1*1104,DQB1*0501	Lamonaca, 1999
aa 2268-2282	NS5a	VSVPAEILRK SRRFA	DRB1*1101	Godkin, 2001
aa 2571-2590	NS5b	KGGRKPARLIVFPDLGVRVC	DRB1*0404,DRB1*0407	Day, 2002
aa 2841-2860	NS5b	ARMILMTHFFSVLIARDQLE	DRB1*1101	Day, 2002
aa 2941-2955	NS5b	CGKYLFNWAV RTKLK	DRB1*1101	Godkin, 2001
aa 2941-2960	NS5b	CGKYLFNWAVRTKLKLTPIA	DRB1*1101	Day, 2002

The NS3 protein has been shown to be one dominant target of CD4+ T cell responses in humans clearing HCV infection[10]. Some epitopes in NS3 have been identified in both humans and chimps with resolved infection (see below)[11-14]. Numerous studies indicate that CD4+ T cells targeting most HCV proteins, including non-structural (NS) proteins, are the norm in self-limited infections[3-6,10-11,15-17,]. However, some caution is needed in interpreting the exact targeting as the genomic variation between different HCV genotypes is substantial and relatively non-conserved epitopes (as might occur in envelope genes) may not always be picked up in such screens.

The number of CD4+ T cell epitopes recognised during acute HCV infection has been estimated by characterising memory CD4+ T cell populations in blood after permanent resolution of the virus: one study showed at least four, and up to 14 epitopes from the core, NS3, NS4 and NS5 proteins were recognised by CD4+ T cells in patients several months or even years after loss of HCV RNA[11]. A recent study identified 13 CD4+ T cell epitopes within the NS3-NS4 region that were recognised by $\geqslant$ 30% of patients with acute or resolved HCV[18]. Of these, eight peptides were also recognised recurrently from different donors by specific CD4+ T cell clones in independent cloning procedures. Multispecific CD4+ T cell responses were also detectable in blood of individuals during acute HCV infection acquired by needlestick injury[6] or Ⅳ drug use[15]. Importantly, in some patients whose infection became chronic after the acute phase infection, responses were similar to those who spontaneously cleared the virus after acute infection[6]. The only difference (as above) was that these responses in individuals with chronic infection were not sustained.

A recent study identified a single epitope restricted by HLA DR1 (a common HLA molecule) in NS4, which appears to be very commonly targeted in infected individuals[18] (Table 1). New analysis using Class Ⅱ tetramers revealed that in acute infection, all DR1+ donors made a response to this peptide[19]. This highly reproducible response is somewhat unusual, but may reflect the fact that this peptide is extremely conserved. Although the responses at presentation were indistinguishable between those who went on to resolve infection or not, early loss of tetramer+ cells was seen in those with persistent infection. The loss of such cells was not associated with mutation within the epitope and populations of such cells were not found localised in the liver only. Interestingly, when compared to controls in long term chronic infection, some very low level tetramer+ populations could be identified in the absence of proliferative responses. This suggests that specific responses were not deleted entirely, but persist at very low levels and with only limited function.

Table 1 HCV T helper epitopes mapped within a region of 21 amino acids or less. The protein, the sequence and HLA restriction elements of the T helper epitopes are provided.

ASSOCIATION BETWEEN HLA CLASS Ⅱ ALLELES AND INFECTION OUTCOME

It is not yet clear whether responses to specific epitopes are clearly related to outcome. However, the T cell response to peptides derived from the virus is directed by the MHC genotype, and this could have a major influence on the quality of T cell responses. Importantly, some MHC Class Ⅱ alleles in humans have been associated with persistence or resolution of HCV infection. For example, HLA-DRB1*0701 has been shown to be associated with

persistence in patients who were homogeneous in terms of gender, source of infection (genotype 1b) and ethnicity[20]. In contrast, a number of other studies of more mixed populations have shown a strong association between other HLA Class Ⅱ alleles and viral control. These alleles are HLA-DRB1*0101, HLA-DRB1*1101, and HLA-DQB1*0301[21-23].

Although specific HLA alleles have been defined as protective, the link between the possession of a specific allele and the presentation of a specific peptide or set of peptides has not yet been made. HLA-DRB1*1101 and HLA-DQB1*0301 (these genes are in close linkage disequilibrium) have been associated with a sustained $CD4^+$ T cell response in resolved HCV infection; these responses were stronger than in non-DQB1*0301+ controls[24]. However, our understanding of this association still remains incomplete. Some of these HLA Class Ⅱ restricted peptides have been identified through epitope prediction programs[25], and the full repertoire of naturally presented peptides is not completely defined. A recent study showed that viral variation might play a role in determining the dominance of epitopes seen within a population. Here, an HLA DR11-restricted epitope (NS3 aa1248-1261) that is highly conserved within viral genotypes was found not to be the immunodominant response, despite being the most commonly recognised epitope for this HLA allele[26]. Epitopes in viral regions that can tolerate amino acid substitutions may thus appear to be less dominant, even if they are important. This is because their capacity for increased variability means that they may not occur in the prototype viral peptide sequences often used for immunological study, or might only be represented in a fraction of the patients studied.

$CD4^+$ T CELL QUALITY AND ASSOCIATION WITH ACUTE OUTCOME

It has been suggested in studies of acute disease that viral clearance is more likely to occur when HCV specific responses of patients display a Th1 profile (IFN-γ and IL-2). Those with a more typical Th2 profile (IL-4 and IL-10) were more likely to become chronic[27] suggesting that the Th1 phenotype generates more protective immune responses in HCV. In that study, $CD4^+$ T-cell proliferation and cytokine secretion in response to a panel of recombinant HCV antigens were assayed in 17 patients with acute HCV. All six patients with self-limited disease had a significant $CD4^+$ T-cell proliferation to C22, E1, C100, C200, and NS5, running parallel with the antigen-stimulated secretion of IL-2 and IFN-γ, but not with IL-4 and IL-10, indicating predominant Th1 responses. Among the remaining 11 patients who developed chronicity, several cases showed specific $CD4^+$ T cell responses, but their antigen-stimulated IL-2 and IFN-γ production were significantly lower than those of cases with recovery. Importantly, IL-4 and IL-10 (Th2 responses) were detectable in the group who developed chronicity. The data suggested that activation of Th2 responses in acute hepatitis C patients might play a role in the development of chronicity, but much more work needs to be done in this area, and why such responses might differ at the start of the disease needs some explanation.

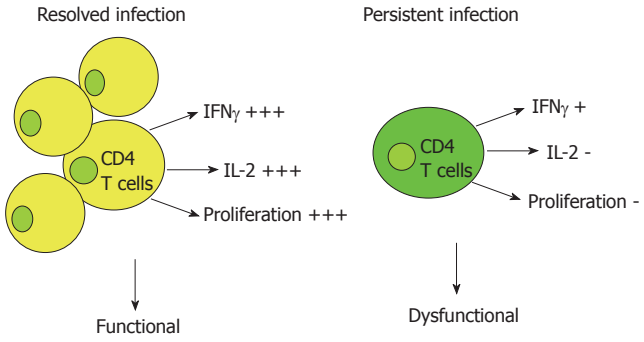

Figure 1 Cytokine secretion patterns in persistent versus resolved HCV infection.

HCV SPECIFIC RESPONSES IN CHRONIC INFECTION

As discussed above, once chronic HCV infection is established, cellular immune responses are rarely or barely detectable using current technology. Several groups have aimed to analyse these HCV-specific $CD4^+$ T cell responses during chronic HCV infection using proliferation assays, cytokine assays and, more recently Class Ⅱ tetramers.

Analysis of HCV-specific $CD4^+$ T cell responses in chronic HCV infection using ELISpot or ICS showed responses at low frequency in blood and only targeted a limited number of epitopes[4,7,11,15-17,28]. In an expanded analysis of responses to recombinant HCV proteins in persons with resolved infection, an average of 10 epitopes was targeted, whereas in persons with chronic viraemia never more than one epitope was targeted[11].

The question of whether HCV-specific $CD4^+$ T cell responses persist in chronic infection but lack function in terms of proliferation and cytokine production (IFN-γ) has been addressed by staining for expression of the IL-2 receptor α-chain (CD25), which is an early marker of activation in a study that showed that a small proportion of $CD4^+$ T cells from the blood of chronically infected individuals did upregulate CD25 after stimulation with recombinant HCV proteins, but lacked the capability for proliferation and IFN-γ production[7]. However, the use of the marker CD25, which is present normally on a fraction of $CD4^+$ T cells including Tregs, limits the usefulness of this assay.

It has generally been assumed that loss of proliferation equates to loss of the specific T cell populations. Low levels of IL-2 secretion have been shown in independent studies in humans and mice to be accompanied by a loss of proliferative capacity both *in vitro* and *in vivo*[29-32]. In HCV infection, recent data suggest that, in the presence of viraemia, HCV-specific $CD4^+$ T cell populations do exist but lack proliferative capacity[33]. This status is associated with the production of IFN-γ upon antigen stimulation, but little or no IL-2 is expressed[34] (Figure 1).

The best approach for detecting functionally impaired

$CD4^+$ T cells in chronic HCV infection is the use of MHC Class Ⅱ tetramers. Using this technique, a correlation has been shown between the clinical outcome and the presence of circulating $CD4^+$ T cells directed against the virus[35]. Here, with the use of 3 HCV HLA Class Ⅱ tetramers, HCV-specific $CD4^+$ T cells could be detected in subjects who spontaneously resolved HCV viraemia, but not in those with chronic HCV infection, suggesting that HCV-specific $CD4^+$ T cell frequencies are very low in PBMC. A further application of this technology for the analysis of intrahepatic $CD4^+$ T cells could shed important light on their differentiation state and functionality.

The expansion of HCV-specific $CD4^+$ T cell lines by repeated stimulation with recombinant antigens indicated that antigen-specific populations do persist[36-38]. A note of caution should be injected here in the analysis of sustained $CD4^+$ T cell responses in chronically infected patients. In many cases, these may represent a historical response to a previous viral genotype which is no longer circulating in the patient. In one case described, the response was directed against genotype 1 even though the patient carried genotype 3, with a historical genotype 1 infection[39]. Since the peptide in the genotype 3 sequence was substantially different and not recognised by host $CD4^+$ T cells, this indicates that the detected response is effectively a memory response after removal of the original virus. Since superinfection is relatively common, this issue may be a substantial one for both $CD4^+$ and $CD8^+$ T cells[40].

The role of IL-10 as Th2 anti-inflammatory cytokine has been demonstrated *in vivo* in humans chronically infected with HCV[41]. Here, individuals with advanced fibrosis were treated three times a week with IL-10 for 12 mo. Administration of IL-10 resulted in a decreased number of IFN-γ-secreting HCV-specific $CD4^+$ and $CD8^+$ T cells. At the same time ALT levels as a marker of inflammation were reduced, indicating the role of IL-10 as anti-inflammatory cytokine. However, with the loss of specific $CD4^+$ and $CD8^+$ T cells, HCV RNA levels were increased, suggesting that these same cells are responsible for viral control[41].

One hypothesis why in chronic HCV the Th2 type may occur is that dendritic cells from patients with chronic HCV infection have defective function, possibly due to inhibition of IL-12[42,43]. The latter cytokine is required for the induction of Th1 type cells. This dendritic cell dysfunction might result in biased T cell polarisation which could favor, for example, a Th2-type response. However, to what extent this is a major factor in pathogenesis is still controversial and the findings are not uniformly reproducible.

Overall, failure of $CD4^+$ T cells is a key factor in HCV persistence and clearly in chronic disease there are relatively few functional $CD4^+$ T cells to find, by whatever method. To some extent this appears to be due to loss/deletion of antigen-specific cells. On the other hand there is some evidence that a change in function also occurs in persistent infection, although whether this is cause or effect requires a great deal more study. It should be noted that although $CD4^+$ T cell responses are regarded as weak in chronic HCV mono-infection, they are even weaker in HCV/HIV co-infection[44,45]. Since co-infection is associated with an increase of HCV load of about 0.5-1 log, this data suggests that in chronic mono-infection the remaining $CD4^+$ T cell response is still playing a significant role.

MODELS FOR FAILURE OF $CD4^+$ T CELL RESPONSES

If failure of the $CD4^+$ T cell response against HCV is associated with virus persistence, what mechanisms could account for this? Here we outline three major contenders, escape, exhaustion and regulation.

Escape through mutation

Numerous studies in both animal and human models have documented immune escape from virus-specific CTL responses by viral mutations in CTL epitopes that lead to loss of immune control and viral persistence[46-52]. Less information is currently available about the potential for immune escape from viral $CD4^+$ T cell epitopes, although limited studies in chronic HIV and HCV infection have identified multiple autologous virus variants for specific $CD4^+$ T cell epitopes[53-56]. Peptides corresponding to viral variants were synthesised and tested in *in vitro* assays, and the majority of variants failed to stimulate proliferation or cytokine production by $CD4^+$ T cells[54-56]. That viral variants may play a role in HCV persistence has also been shown previously in a study with four HLA-DRB1*15 patients chronically infected with HCV[57]. Here, naturally occurring single amino acid substitutions in the DRB1*15-restricted Th1 epitope (aa 358-375) in the NS3 protein failed to stimulate proliferation. This was also accompanied by a shift in cytokine secretion patterns from one characteristic of a Th1 anti-viral response to a Th2 form. These data suggest that viral immune escape from specific $CD4^+$ T cell responses is possible, but clear data showing the evolution of $CD4^+$ T cell escape mutants in response to T cell selection pressure are still needed.

A recent study analyzed the effects of an induced T-cell response in three immunized chimpanzees, targeting nonstructural proteins in the absence of neutralizing antibodies[58]. The immunized animals were challenged with clonal HCV, which had the same sequence as the antigens used for immunization. Persistent control of the virus was observed in two animals, whereas in the third animal viral control was transient, followed by a resurgence concomitant with the emergence of new dominant viral populations bearing single amino acid changes in the NS3 and NS5A regions. These mutations resulted in a loss of CD4 T-cell recognition and subsequent to viral resurgence and immune escape a large fraction of NS3-specific T cells became impaired in their ability to secrete IFN-γ and proliferate.

Exhaustion

In addition to escape from virus-specific T cell responses, escape from neutralising antibody (nAb) responses is thought to be one potential mechanism leading to the persistence of some viruses with knock on effects on $CD4^+$ T cells[53,59,60]. Recent data generated in an LCMV model have provided additional insight into the relationship between

$CD4^+$ T cells and immune escape from nAb responses. $CD8^{-/-}$ mice were infected with the WE strain of LCMV to establish a long-term infection with high levels of virus production that is transiently controlled by nAbs[60]. However, the lack of $CD8^+$ CTL responses and consequently high viraemia in this model leads to escape from polyclonal nAb responses. Associated with this is the rapid induction of $CD4^+$ T cell unresponsiveness[61]. Although the molecular mechanism of $CD4^+$ T cell unresponsiveness is not clear from this study, it has been postulated that the high antigenic load in this model system may have resulted in activation of all virus-specific $CD4^+$ T cells, leading to exhaustion and activation-induced cell death as has been described for CTLs[62,63]. Importantly, in the absence of LCMV-specific $CD4^+$ T cells, these mice failed to generate new effective humoral responses against emerging neutralisation-escape mutants and the viral infection persisted[61]. These data provide further evidence for the importance of interactions between the cellular and humoral immune responses for efficient control of viral infections. A similar phenomenon of exhaustion of $CD4^+$ T cells could easily arise through any mechanism, which leads to long-term viremia, including escape from interferon or mutation in epitopes recognised by $CD8^+$ T cells.

Regulation

Recent years have seen a revival of interest in the role of regulatory T cells-notably the $CD4^+$ $CD25^+$ FoxP3+ subset[64]. It is plausible that in HCV infection excessive regulation is involved in the suppression of HCV specific T-cell responses. Recently, $CD4^+$ $CD25^+$ regulatory T-cell activity has been shown to be present in patients with chronic HCV infection, which may contribute to weak HCV-specific T-cell responses and viral persistence[65-69]. An important question that derives from these studies is to what extent the Treg activity seen in persistent infection relates to the activity of antigen-specific cells. Treg cells may arise in the thymus (natural Tregs) but additionally virus-specific cells, which are repetitively stimulated with antigen over time, may develop regulatory characteristics. This activity may be promoted by the action of dendritic cell subsets modulated by persistent viremia. Such Treg cells might serve to downregulate both $CD4^+$ and $CD8^+$ T cell responses in persistent infection, particularly within the inflamed liver. A recent study revealed that approximately one in two to one in three $CD4^+$ T cells in the liver of chronically infected patients are FoxP3+, a remarkably dominant potential Treg population[70]. This could have a major effect on the maintenance and growth of antigen-specific $CD4^+$ and $CD8^+$ T cells.

CONCLUSION

HCV specific $CD4^+$ T cells hold a pivotal role in disease pathogenesis. There is a consensus that there are real differences between the responses seen in resolved infection *vs* persistent infection, although to what extent these are cause or consequence is not clear yet. The differences include not only number, but also function, including cytokine secretion, such as the key mediator IL-2. A number of pieces of evidence point to the fact that a robust $CD4^+$ T cell response is associated with a good outcome from acute infection and there is no doubt that such responses should be elicited in a vaccine. To what extent it matters which epitopes are targeted or not is not yet clear, but most observers argue that breadth is important, especially given the huge genomic variation in HCV, and that numbers are important. For those lucky enough to inherit protective HLA types, a vaccine may simply augment an already favourable response and sustain adaptive responses from $CD8^+$ and B cells. For the rest, perhaps a pool of $CD4^+$ T cell responses which are primed in a normal uninfected individual, and which can expand rapidly during acute infection may be sufficient to tip the balance in favour of host clearance.

REFERENCES

1 **Gremion C**, Cerny A. Hepatitis C virus and the immune system: a concise review. *Rev Med Virol* 2005; **15**: 235-**268**
2 **Grakoui A**, Shoukry NH, Woollard DJ, Han JH, Hanson HL, Ghrayeb J, Murthy KK, Rice CM, Walker CM. HCV persistence and immune evasion in the absence of memory T cell help. *Science* 2003; **302**: 659-**662**
3 **Thimme R**, Bukh J, Spangenberg HC, Wieland S, Pemberton J, Steiger C, Govindarajan S, Purcell RH, Chisari FV. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. *Proc Natl Acad Sci USA* 2002; **99**: 15661-**15668**
4 **Gerlach JT**, Diepolder HM, Jung MC, Gruener NH, Schraut WW, Zachoval R, Hoffmann R, Schirren CA, Santantonio T, Pape GR. Recurrence of hepatitis C virus after loss of virus-specific CD4(+) T-cell response in acute hepatitis C. *Gastroenterology* 1999; **117**: 933-**941**
5 **Missale G**, Bertoni R, Lamonaca V, Valli A, Massari M, Mori C, Rumi MG, Houghton M, Fiaccadori F, Ferrari C. Different clinical behaviors of acute hepatitis C virus infection are associated with different vigor of the anti-viral cell-mediated immune response. *J Clin Invest* 1996; **98**: 706-**714**
6 **Thimme R**, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. *J Exp Med* 2001; **194**: 1395-**1406**
7 **Ulsenheimer A**, Gerlach JT, Gruener NH, Jung MC, Schirren CA, Schraut W, Zachoval R, Pape GR, Diepolder HM. Detection of functionally altered hepatitis C virus-specific CD4 T cells in acute and chronic hepatitis C. *Hepatology* 2003; **37**: 1189-**1198**
8 **Ulsenheimer A**, Lucas M, Seth NP, Tilman Gerlach J, Gruener NH, Loughry A, Pape GR, Wucherpfennig KW, Diepolder HM, Klenerman P. Transient immunological control during acute hepatitis C virus infection: ex vivo analysis of helper T-cell responses. *J Viral Hepat* 2006; **13**: 708-**714**
9 **Aberle JH**, Formann E, Steindl-Munda P, Weseslindtner L, Gurguta C, Perstinger G, Grilnberger E, Laferl H, Dienes HP, Popow-Kraupp T, Ferenci P, Holzmann H. Prospective study of viral clearance and CD4(+) T-cell response in acute hepatitis C primary infection and reinfection. *J Clin Virol* 2006; **36**: 24-31
10 **Diepolder HM**, Zachoval R, Hoffmann RM, Wierenga EA, Santantonio T, Jung MC, Eichenlaub D, Pape GR. Possible mechanism involving T-lymphocyte response to non-structural protein 3 in viral clearance in acute hepatitis C virus infection. *Lancet* 1995; **346**: 1006-**1007**
11 **Day CL**, Lauer GM, Robbins GK, McGovern B, Wurcel AG, Gandhi RT, Chung RT, Walker BD. Broad specificity of virus-specific CD4+ T-helper-cell responses in resolved hepatitis C virus infection. *J Virol* 2002; **76**: 12584-**12595**
12 **Lamonaca V**, Missale G, Urbani S, Pilli M, Boni C, Mori C, Sette A, Massari M, Southwood S, Bertoni R, Valli A, Fiaccadori F, Ferrari C. Conserved hepatitis C virus sequences are highly immunogenic for CD4(+) T cells: implications for vac-

cine development. *Hepatology* 1999; **30**: 1088-**1098**
13 **Shoukry NH**, Sidney J, Sette A, Walker CM. Conserved hierarchy of helper T cell responses in a chimpanzee during primary and secondary hepatitis C virus infections. *J Immunol* 2004; **172**: 483-**492**
14 **Diepolder HM**, Gerlach JT, Zachoval R, Hoffmann RM, Jung MC, Wierenga EA, Scholz S, Santantonio T, Houghton M, Southwood S, Sette A, Pape GR. Immunodominant CD4+ T-cell epitope within nonstructural protein 3 in acute hepatitis C virus infection. *J Virol* 1997; **71**: 6011-**6019**
15 **Lechner F**, Wong DK, Dunbar PR, Chapman R, Chung RT, Dohrenwend P, Robbins G, Phillips R, Klenerman P, Walker BD. Analysis of successful immune responses in persons infected with hepatitis C virus. *J Exp Med* 2000; **191**: 1499-**1512**
16 **Rosen HR**, Miner C, Sasaki AW, Lewinsohn DM, Conrad AJ, Bakke A, Bouwer HG, Hinrichs DJ. Frequencies of HCV-specific effector CD4+ T cells by flow cytometry: correlation with clinical disease stages. *Hepatology* 2002; **35**: 190-**198**
17 **Takaki A**, Wiese M, Maertens G, Depla E, Seifert U, Liebetrau A, Miller JL, Manns MP, Rehermann B. Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis C. *Nat Med* 2000; **6**: 578-**582**
18 **Gerlach JT**, Ulsenheimer A, Grüner NH, Jung MC, Schraut W, Schirren CA, Heeg M, Scholz S, Witter K, Zahn R, Vogler A, Zachoval R, Pape GR, Diepolder HM. Minimal T-cell-stimulatory sequences and spectrum of HLA restriction of immunodominant CD4+ T-cell epitopes within hepatitis C virus NS3 and NS4 proteins. *J Virol* 2005; **79**: 12425-**12433**
19 **Lucas M**, Ulsenheimer A, Pfafferot K, Heeg MH, Gaudieri S, Grüner N, Rauch A, Gerlach JT, Jung MC, Zachoval R, Pape GR, Schraut W, Santantonio T, Nitschko H, Obermeier M, Phillips R, Scriba TJ, Semmo N, Day C, Weber JN, Fidler S, Thimme R, Haberstroh A, Baumert TF, Klenerman P, Diepolder HM. Tracking virus-specific CD4+ T cells during and after acute hepatitis C virus infection. *PLoS ONE* 2007; **2**: e6**49**
20 **Fanning LJ**, Levis J, Kenny-Walsh E, Whelton M, O'Sullivan K, Shanahan F. HLA class II genes determine the natural variance of hepatitis C viral load. *Hepatology* 2001; **33**: 224-**230**
21 **Alric L**, Fort M, Izopet J, Vinel JP, Charlet JP, Selves J, Puel J, Pascal JP, Duffaut M, Abbal M. Genes of the major histocompatibility complex class II influence the outcome of hepatitis C virus infection. *Gastroenterology* 1997; **113**: 1675-**1681**
22 **Minton EJ**, Smillie D, Neal KR, Irving WL, Underwood JC, James V. Association between MHC class II alleles and clearance of circulating hepatitis C virus. Members of the Trent Hepatitis C Virus Study Group. *J Infect Dis* 1998; **178**: 39-44
23 **Thursz M**, Yallop R, Goldin R, Trepo C, Thomas HC. Influence of MHC class II genotype on outcome of infection with hepatitis C virus. The HENCORE group. Hepatitis C European Network for Cooperative Research. *Lancet* 1999; **354**: 2119-**2124**
24 **Harcourt G**, Hellier S, Bunce M, Satsangi J, Collier J, Chapman R, Phillips R, Klenerman P. Effect of HLA class II genotype on T helper lymphocyte responses and viral control in hepatitis C virus infection. *J Viral Hepat* 2001; **8**: 174-**179**
25 **Godkin A**, Jeanguet N, Thursz M, Openshaw P, Thomas H. Characterization of novel HLA-DR11-restricted HCV epitopes reveals both qualitative and quantitative differences in HCV-specific CD4+ T cell responses in chronically infected and non-viremic patients. *Eur J Immunol* 2001; **31**: 1438-**1446**
26 **Harcourt GC**, Lucas M, Sheridan I, Barnes E, Phillips R, Klenerman P. Longitudinal mapping of protective CD4+ T cell responses against HCV: analysis of fluctuating dominant and subdominant HLA-DR11 restricted epitopes. *J Viral Hepat* 2004; **11**: 324-**331**
27 **Tsai SL**, Liaw YF, Chen MH, Huang CY, Kuo GC. Detection of type 2-like T-helper cells in hepatitis C virus infection: implications for hepatitis C virus chronicity. *Hepatology* 1997; **25**: 449-**458**
28 **Wertheimer AM**, Miner C, Lewinsohn DM, Sasaki AW, Kaufman E, Rosen HR. Novel CD4+ and CD8+ T-cell determinants within the NS3 protein in subjects with spontaneously resolved HCV infection. *Hepatology* 2003; **37**: 577-**589**
29 **Younes SA**, Yassine-Diab B, Dumont AR, Boulassel MR, Grossman Z, Routy JP, Sekaly RP. HIV-1 viremia prevents the establishment of interleukin 2-producing HIV-specific memory CD4+ T cells endowed with proliferative capacity. *J Exp Med* 2003; **198**: 1909-**1922**
30 **Iyasere C**, Tilton JC, Johnson AJ, Younes S, Yassine-Diab B, Sekaly RP, Kwok WW, Migueles SA, Laborico AC, Shupert WL, Hallahan CW, Davey RT Jr, Dybul M, Vogel S, Metcalf J, Connors M. Diminished proliferation of human immunodeficiency virus-specific CD4+ T cells is associated with diminished interleukin-2 (IL-2) production and is recovered by exogenous IL-2. *J Virol* 2003; **77**: 10900-**10909**
31 **Wherry EJ**, Teichgräber V, Becker TC, Masopust D, Kaech SM, Antia R, von Andrian UH, Ahmed R. Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat Immunol* 2003; **4**: 225-**234**
32 **Fuller MJ**, Zajac AJ. Ablation of CD8 and CD4 T cell responses by high viral loads. *J Immunol* 2003; **170**: 477-**486**
33 **Semmo N**, Krashias G, Willberg C, Klenerman P. Analysis of the relationship between cytokine secretion and proliferative capacity in hepatitis C virus infection. *J Viral Hepat* 2007; **14**: 492-502
34 **Semmo N**, Day CL, Ward SM, Lucas M, Harcourt G, Loughry A, Klenerman P. Preferential loss of IL-2-secreting CD4+ T helper cells in chronic HCV infection. *Hepatology* 2005; **41**: 1019-**1028**
35 **Day CL**, Seth NP, Lucas M, Appel H, Gauthier L, Lauer GM, Robbins GK, Szczepiorkowski ZM, Casson DR, Chung RT, Bell S, Harcourt G, Walker BD, Klenerman P, Wucherpfennig KW. Ex vivo analysis of human memory CD4 T cells specific for hepatitis C virus using MHC class II tetramers. *J Clin Invest* 2003; **112**: 831-**842**
36 **Minutello MA**, Pileri P, Unutmaz D, Censini S, Kuo G, Houghton M, Brunetto MR, Bonino F, Abrignani S. Compartmentalization of T lymphocytes to the site of disease: intrahepatic CD4+ T cells specific for the protein NS4 of hepatitis C virus in patients with chronic hepatitis C. *J Exp Med* 1993; **178**: 17-25
37 **Penna A**, Missale G, Lamonaca V, Pilli M, Mori C, Zanelli P, Cavalli A, Elia G, Ferrari C. Intrahepatic and circulating HLA class II-restricted, hepatitis C virus-specific T cells: functional characterization in patients with chronic hepatitis C. *Hepatology* 2002; **35**: 1225-**1236**
38 **Schirren CA**, Jung MC, Gerlach JT, Worzfeld T, Baretton G, Mamin M, Hubert Gruener N, Houghton M, Pape GR. Liver-derived hepatitis C virus (HCV)-specific CD4(+) T cells recognize multiple HCV epitopes and produce interferon gamma. *Hepatology* 2000; **32**: 597-603
39 **Harcourt GC**, Lucas M, Godkin AJ, Kantzanou M, Phillips RE, Klenerman P. Evidence for lack of cross-genotype protection of CD4+ T cell responses during chronic hepatitis C virus infection. *Clin Exp Immunol* 2003; **131**: 122-**129**
40 **Lauer GM**, Barnes E, Lucas M, Timm J, Ouchi K, Kim AY, Day CL, Robbins GK, Casson DR, Reiser M, Dusheiko G, Allen TM, Chung RT, Walker BD, Klenerman P. High resolution analysis of cellular immune responses in resolved and persistent hepatitis C virus infection. *Gastroenterology* 2004; **127**: 924-**936**
41 **Nelson DR**, Tu Z, Soldevila-Pico C, Abdelmalek M, Zhu H, Xu YL, Cabrera R, Liu C, Davis GL. Long-term interleukin 10 therapy in chronic hepatitis C patients has a proviral and anti-inflammatory effect. *Hepatology* 2003; **38**: 859-**868**
42 **Bain C**, Fatmi A, Zoulim F, Zarski JP, Trépo C, Inchauspé G. Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection. *Gastroenterology* 2001; **120**: 512-**524**
43 **Fowler NL**, Torresi J, Jackson DC, Brown LE, Gowans EJ. Immune responses in hepatitis C virus infection: the role of dendritic cells. *Immunol Cell Biol* 2003; **81**: 63-**66**
44 **Lauer GM**, Nguyen TN, Day CL, Robbins GK, Flynn T, McGowan K, Rosenberg ES, Lucas M, Klenerman P, Chung RT, Walker BD. Human immunodeficiency virus type 1-hepatitis C virus coinfection: intraindividual comparison of cellular immune responses against two persistent viruses. *J Virol* 2002; **76**: 2817-**2826**

45 **Harcourt G**, Gomperts E, Donfield S, Klenerman P. Diminished frequency of hepatitis C virus specific interferon {gamma} secreting CD4+ T cells in human immunodeficiency virus/hepatitis C virus coinfected patients. *Gut* 2006; **55**: 1484-**1487**

46 **Erickson AL**, Kimura Y, Igarashi S, Eichelberger J, Houghton M, Sidney J, McKinney D, Sette A, Hughes AL, Walker CM. The outcome of hepatitis C virus infection is predicted by escape mutations in epitopes targeted by cytotoxic T lymphocytes. *Immunity* 2001; **15**: 883-**895**

47 **Vogel TU**, Friedrich TC, O'Connor DH, Rehrauer W, Dodds EJ, Hickman H, Hildebrand W, Sidney J, Sette A, Hughes A, Horton H, Vielhuber K, Rudersdorf R, De Souza IP, Reynolds MR, Allen TM, Wilson N, Watkins DI. Escape in one of two cytotoxic T-lymphocyte epitopes bound by a high-frequency major histocompatibility complex class I molecule, Mamu-A*02: a paradigm for virus evolution and persistence? *J Virol* 2002; **76**: 11623-**11636**

48 **O'Connor DH**, Allen TM, Vogel TU, Jing P, DeSouza IP, Dodds E, Dunphy EJ, Melsaether C, Mothé B, Yamamoto H, Horton H, Wilson N, Hughes AL, Watkins DI. Acute phase cytotoxic T lymphocyte escape is a hallmark of simian immunodeficiency virus infection. *Nat Med* 2002; **8**: 493-**499**

49 **Allen TM**, O'Connor DH, Jing P, Dzuris JL, Mothé BR, Vogel TU, Dunphy E, Liebl ME, Emerson C, Wilson N, Kunstman KJ, Wang X, Allison DB, Hughes AL, Desrosiers RC, Altman JD, Wolinsky SM, Sette A, Watkins DI. Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. *Nature* 2000; **407**: 386-**390**

50 **Altfeld M**, Allen TM, Yu XG, Johnston MN, Agrawal D, Korber BT, Montefiori DC, O'Connor DH, Davis BT, Lee PK, Maier EL, Harlow J, Goulder PJ, Brander C, Rosenberg ES, Walker BD. HIV-1 superinfection despite broad CD8+ T-cell responses containing replication of the primary virus. *Nature* 2002; **420**: 434-**439**

51 **Goulder PJ**, Phillips RE, Colbert RA, McAdam S, Ogg G, Nowak MA, Giangrande P, Luzzi G, Morgan B, Edwards A, McMichael AJ, Rowland-Jones S. Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. *Nat Med* 1997; **3**: 212-**217**

52 **Borrow P**, Lewicki H, Wei X, Horwitz MS, Peffer N, Meyers H, Nelson JA, Gairin JE, Hahn BH, Oldstone MB, Shaw GM. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. *Nat Med* 1997; **3**: 205-**211**

53 **von Hahn T**, Yoon JC, Alter H, Rice CM, Rehermann B, Balfe P, McKeating JA. Hepatitis C virus continuously escapes from neutralizing antibody and T-cell responses during chronic infection in vivo. *Gastroenterology* 2007; **132**: 667-**678**

54 **Wang H**, Eckels DD. Mutations in immunodominant T cell epitopes derived from the nonstructural 3 protein of hepatitis C virus have the potential for generating escape variants that may have important consequences for T cell recognition. *J Immunol* 1999; **162**: 4177-**4183**

55 **Harcourt GC**, Garrard S, Davenport MP, Edwards A, Phillips RE. HIV-1 variation diminishes CD4 T lymphocyte recognition. *J Exp Med* 1998; **188**: 1785-**1793**

56 **Eckels DD**, Zhou H, Bian TH, Wang H. Identification of antigenic escape variants in an immunodominant epitope of hepatitis C virus. *Int Immunol* 1999; **11**: 577-**583**

57 **Wang JH**, Layden TJ, Eckels DD. Modulation of the peripheral T-Cell response by CD4 mutants of hepatitis C virus: transition from a Th1 to a Th2 response. *Hum Immunol* 2003; **64**: 662-**673**

58 **Puig M**, Mihalik K, Tilton JC, Williams O, Merchlinsky M, Connors M, Feinstone SM, Major ME. CD4+ immune escape and subsequent T-cell failure following chimpanzee immunization against hepatitis C virus. *Hepatology* 2006; **44**: 736-**745**

59 **Parren PW**, Moore JP, Burton DR, Sattentau QJ. The neutralizing antibody response to HIV-1: viral evasion and escape from humoral immunity. *AIDS* 1999; **13** Suppl A: S137-**S162**

60 **Ciurea A**, Klenerman P, Hunziker L, Horvath E, Senn BM, Ochsenbein AF, Hengartner H, Zinkernagel RM. Viral persistence in vivo through selection of neutralizing antibody-escape variants. *Proc Natl Acad Sci USA* 2000; **97**: 2749-**2754**

61 **Ciurea A**, Hunziker L, Klenerman P, Hengartner H, Zinkernagel RM. Impairment of CD4(+) T cell responses during chronic virus infection prevents neutralizing antibody responses against virus escape mutants. *J Exp Med* 2001; **193**: 297-305

62 **Gallimore A**, Glithero A, Godkin A, Tissot AC, Plückthun A, Elliott T, Hengartner H, Zinkernagel R. Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. *J Exp Med* 1998; **187**: 1383-**1393**

63 **Moskophidis D**, Lechner F, Pircher H, Zinkernagel RM. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. *Nature* 1993; **362**: 758-**761**

64 **Rouse BT**, Sarangi PP, Suvas S. Regulatory T cells in virus infections. *Immunol Rev* 2006; **212**: 272-**286**

65 **Rushbrook SM**, Ward SM, Unitt E, Vowler SL, Lucas M, Klenerman P, Alexander GJ. Regulatory T cells suppress in vitro proliferation of virus-specific CD8+ T cells during persistent hepatitis C virus infection. *J Virol* 2005; **79**: 7852-**7859**

66 **Boettler T**, Spangenberg HC, Neumann-Haefelin C, Panther E, Urbani S, Ferrari C, Blum HE, von Weizsäcker F, Thimme R. T cells with a CD4+CD25+ regulatory phenotype suppress in vitro proliferation of virus-specific CD8+ T cells during chronic hepatitis C virus infection. *J Virol* 2005; **79**: 7860-**7867**

67 **Cabrera R**, Tu Z, Xu Y, Firpi RJ, Rosen HR, Liu C, Nelson DR. An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. *Hepatology* 2004; **40**: 1062-**1071**

68 **Sugimoto K**, Ikeda F, Stadanlick J, Nunes FA, Alter HJ, Chang KM. Suppression of HCV-specific T cells without differential hierarchy demonstrated ex vivo in persistent HCV infection. *Hepatology* 2003; **38**: 1437-**1448**

69 **Bolacchi F**, Sinistro A, Ciaprini C, Demin F, Capozzi M, Carducci FC, Drapeau CM, Rocchi G, Bergamini A. Increased hepatitis C virus (HCV)-specific CD4+CD25+ regulatory T lymphocytes and reduced HCV-specific CD4+ T cell response in HCV-infected patients with normal versus abnormal alanine aminotransferase levels. *Clin Exp Immunol* 2006; **144**: 188-**196**

70 **Ward SM**, Fox BC, Brown PJ, Worthington J, Fox SB, Chapman RW, Fleming KA, Banham AH, Klenerman P. Quantification and localisation of FOXP3+ T lymphocytes and relation to hepatic inflammation during chronic HCV infection. *J Hepatol* 2007; **47**: 316-**324**

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