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Review

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Immunology of viral co-infections with HIV

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Summary

Increasing clinical evidence is emerging that other persistent viral infections can act as important co-factors affecting the progression of human immunodeficiency virus-1 (HIV-1). It appears that hepatitis C (HCV) and cytomegalovirus (CMV) have a deleterious effect on HIV progression, whereas hepatitis G (GBV-C) benefits HIV-1 progression. At the same time, the aggressive nature of HCV infection in HIV is clearly recognized. Here we discuss this clinical evidence and go on to review scientific work pertaining to these interactions in the context of the known and theoretical immunological effects of these viruses. This is discussed at the level of the generation of adaptive immune responses and their effector functions. It is clear that co-infection with persistent viral infections may pose special problems for the human immune system, as pathogenic effects may not be specific to the actual eliciting virus and can therefore multiply the difficulties faced by host defenses. We also highlight the need for further therapies for HIV/HCV co-infected persons, as this is currently a complex and severe syndrome.

Key words: **HIV • GBV-C • CMV • hepatitis C • T cell • dendritic cell**

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INTRODUCTION: THE CLINICAL PROBLEM

Human immunodeficiency virus (HIV) and hepatitis C virus (HCV) co-infection is common, with HCV infection affecting up to 80% of intravenous drug users, 98% of hemophiliacs, and 2.6–15.2% of homosexual men who have HIV-1[46]. Progression of HCV--related liver disease is more rapid and HCV viral load is higher in those with HIV-1[44, 46]. With the relative success of highly active anti-retroviral therapy (HAART), HCV may now account for up to 50% of the deaths in co-infected subjects. Clearance of HCV following acute infection (which usually occurs in 20% of mono-infected individuals) could be impaired in HIV-1-infected individuals[69], and HIV-1 infection also favors vertical transmission of HCV from mother to child.

HCV may adversely affect the progression of HIV

There is some evidence that HCV may in turn adversely affect the prognosis of HIV. However, detection of such an effect is difficult because of confounding factors, such as differences in treatment for HIV because of compliance and hepatotoxicity issues in co-infected individuals. The Swiss Cohort Study (SCGS) enrolled 3655 HIV-infected subjects commencing HAART (37.2% of whom were also co-infected with HCV) and the relative risk of an AIDS-defining illness or death was 1.7 (CI 1.26-2.3) in HCV^+ persons compared with HCV^- persons[26]. Importantly, this effect was independent of HIV viral load during HAART, active continuation of intravenous drug abuse (which may be related to poor compliance with therapy), and the number of changes to HAART during follow-up, suggesting that compliance and hepatotoxicity issues did not confound the result of this study. The SCGS eclipsed a large study from Spain that corroborated its findings. This, however, was published only in the form of a letter[64]. In a further study of co-infection in persons with hemophilia[16], a "dose-response" relationship for burden of HCV infection and HIV progression was found. With each ten-fold increase in HCV viral load, a relative risk of progression to AIDS of 1.66 (95% CI 1.10-2.51) was seen. Importantly, this was reported to be independent of HIV viral load and $CD4^+$ T cell count. Further evidence suggests that HCV infection induces a defect in immune reconstitution in response to HAART, as the rise in $CD4^+$ count in response to HAART is blunted in co-infected subjects[26, 43, 64].

These findings were not corroborated by several other studies, notably a US-based study by Sulkowski et al.[68], which recruited 1955 HIV-infected individuals, 44.6% of whom were also HCV infected. Unlike the SCGS study, however, the median duration of follow-up was shorter and 72% of patients were not receiving HAART. Subsequently, only 208 patients attained undetectable viral load, 25% of patients developed AIDS, and approximately 16% of patients died, compared with a 6% risk of progression to AIDS and 5% risk of death in the SCGS. Thus the progression to AIDS was rapid and therefore this study may have been unable to detect a late and subtle influence of HCV. Finally, the demographics of the two populations were quite different in the two studies as most patients were of African-American origin in the US study, and it is plausible that the effect of HCV may be determined to some extent by underlying factors such as tissue type. Rancinan et al.[53] also failed to find an effect of HCV on HIV progression, although this study only enrolled 600 HCV^+ persons. In a further study by Tedaldi et al.[72], HCV was discounted as a risk factor for progression of HIV once correction for baseline $CD4^+$ count was performed, but HCV^+/HIV^+ co-infected persons had lower $CD4^+$ counts at baseline than HIV mono-infected persons at the initiation of the study, as has been reported elsewhere[64, 68]. Adjustment for baseline $CD4^+$ count could be misleading as, paradoxically, it might adjust for the presence of HCV itself. Also this study excluded persons who had acquired HCV prior to 1996, and followed cases up for only 3 years or less.

The overall balance of evidence is therefore consistent with at least a mild effect of HCV on HIV progression. The possible immunological basis for this will be reviewed here.

Other important co-infections – CMV and GBV-C

There is also evidence that cytomegalovirus (CMV) accelerates HIV replication and increases the rate of progression to AIDS (see later). An intriguing relationship between HIV-1 and hepatitis G virus (GBV--C) has also been recognized[86]. GBV-C virus is closely related to HCV but is not associated with any morbidity itself. It replicates in lymphocytes and not hepatocytes, and chronic viremia may persist for many years. Active infection with GBV-C is protective in HIV and is associated with a reduced risk of death. However, paradoxically, clearance of GBV-C RNA and seroconversion is associated with a worse prognosis for HIV than in those with no evidence of past or present infection with GBV-C, and as yet there is no explanation for this phenomenon. GBV-C virus infection may be more common in HIV/HCV co-infected persons[14], and this may also result in a detrimental influence of HCV being underestimat-

ed. Thus there are clear interactions between HIV and HCV, HIV and CMV, and HIV and GBV-C in terms of clinical outcome. It is likely that immunological mechanisms underlie these observations and a discussion of these underlies the purpose of this review.

HOW DOES HIV WORSEN THE PROGNOSIS OF HCV?

Impairment of immune responses to HCV in HIV--infected subjects is suggested by the finding of elevated HCV viral load in HIV$^+$ persons[2]. The most important defect induced by HIV is likely to be loss of T helper (Th) cells, the hallmark of HIV-mediated immuno-suppression, although other mechanisms may play their part.

Role of loss of CD4$^+$ Th cells

Wide-ranging observations suggest that CD4$^+$ T cells are important in controlling HCV infection:

- in a chimpanzee model of HCV, selective depletion of CD4$^+$ T cells resulted in increased susceptibility to re-infection[25],
- in man, particular MHC class II genes have been strongly associated with spontaneous resolution of disease[12, 73], and possession of one of these alleles is linked to possession of a broader, functional antiviral T cell response[28],
- functional studies have linked CD4$^+$ T cell proliferative responses with disease outcome[46],
- HCV-specific CD4$^+$ T cells have been isolated *ex vivo*, using MHC class II tetramers, in persons who have resolved infection with HCV but not in chronically infected persons[17], suggesting that successful evolution and maintenance of CD4$^+$ T cell responses may play a role in determining outcome in HCV,
- a role for CD4$^+$ cells in HIV/HCV co-infected persons is supported by the finding that CD4 count is positively correlated with immune responses to the HCV peptide and negatively correlated with HCV viral load[76],
- the magnitude and breadth of HCV-specific CD8$^+$ T cell responses, but not those to EBV and HIV itself, has been recently shown to depend on absolute CD4$^+$ T cell count in co-infected individuals[35].

Role of non-classical T cells

Other elements of the immune system, such as γδ T cells, may also interact in co-infection[2]. γδ T cells are a defined group of T cells that do not respond to classical MHC-restricted elements, but may respond to cellular markers of stress, such as MICA and MICB, or non-peptide-derived products of pathogens and show evidence of "memory", with increased responses after second exposure to a pathogen. A subtype of γδ cells, Vδ1 cells, are specifically recruited to the liver in HCV, where they are not seen in other liver diseases. Increased hepatic Vδ1 cell numbers, and increased interferon (IFN)-γ secretion in response to activation by PMA, were associated with greater necro-inflammatory changes in liver biopsies[2].

In HIV infection, Vδ1 cells are found at increased frequencies in peripheral blood, suggesting that in the context of HCV infection, increased recruitment to the liver could occur[2]. γδ Cells may be important in linking immune responses to liver fibrosis, as they produce connective tissue growth factor (CTGF)[87], a fibrogenic stimulus that could be important in liver fibrosis[29]. Importantly, alterations in Vδ1 frequencies were not reversed by HAART, which casts doubt on the ability of HAART to benefit HCV progression with respect to components of the immune system such as γδ cells[2].

Role of loss of neutralizing antibodies

Antibody responses to HCV may be impaired in persons with HIV[69], but the overall *in vivo* influence of HCV antibodies is not clear, as good measures of neutralization are not yet available. Specific antibodies will select for mutations in specific areas of the viral genome, particularly the hypervariable region HVR1.

Our group as well as others have observed a strong selection pressure on the viral envelope glycoprotein E2 protein, which includes sites targeted by antibody. Greater selection during acute infection is associated with increased progression rate of HCV[21, 63]. Interestingly, treatment with HAART did not lead to significant increases in selection pressure in this region[12], but did lead to selection of mutated sites in the core. One possible interpretation of this is that immune reconstitution has a greater effect on cellular immune responses to HCV (which target the core), than on antibody.

Other potentially important interactions

Recently it has been reported that the HIV envelope protein can induce apoptosis of hepatocytes[79]. This may be an important mechanism for additional hepatic injury. Finally, dendritic cells (DCs) are major antigen-presenting cells that might be the focal point of important interactions between HIV and HCV. This is discussed in the section entitled "How might HCV affect progression of HIV".

HAART AND PROGRESSION OF HCV INFECTION

HAART is now the cornerstone of the management of HIV infection. It restores the immune system, reduces the risk of opportunistic infections, and therefore delays progression to AIDS. It is unclear, however, if HAART necessarily reduces the progression of liver disease to the levels seen in HCV^+HIV^- persons. Indeed one study has failed to report a benefit of HAART on liver disease in people with HCV[66]. A rise in HCV viral load on commencing HAART is common[9, 12], suggesting that HAART may not necessarily improve the course of HCV infection, and might actually worsen it in some cases. Some immune defects induced by HIV may not be readily reversed by HAART. These may include effects of HIV on γ/δ cells and on immunoglobulin production, as discussed. In some instances, immune reconstitution following HAART may aggravate HCV-related immunopathology, as a HAART-induced rise in $CD4^+$ T cell count by 50 or more has been independently associated with severe HAART-related hepatotoxicity[70].

Some drugs used as part of HAART may have direct effects on the immune system. For example the protease inhibitor ritonavir has inhibitory effects on T cells responses[5]. Such effects are of theoretical benefit in HIV, by reducing excessive immune activation and immunopathology, but could be deleterious for the more modest and potentially fragile responses seen to HCV (see below). Such an effect could explain, in part, the hepatoxicity of ritonavir in HCV-infected persons. HAART may also play a direct role in aggravating liver disease, as it consists of chronic treatment with a combination of potentially hepatotoxic drugs. Infection with HCV or hepatitis B is an independent risk factor for HAART-induced hepatotoxicity[60]. However, in a recent study[44] multi-variate analysis showed receipt of HAART was not independently associated with progression of HCV, and most people (88%) did not experience noticeable hepatotoxicity[70]. Therapeutic responses to IFN-α, used to treat HCV, appear to be blunted in HCV^+ persons with HIV, a finding that underlies the need for further treatment options in these persons[50].

HOW MIGHT HCV AFFECT PROGRESSION OF HIV?

There are several potential mechanisms by which HCV could influence the progression of HIV. These are not mutually exclusive and may actually reflect consequences of HCV infection from proximal points of the immune cascade, such as antigen presentation.

Impaired antigen presentation in HCV infection

DCs are key antigen-presenting cells in the evolution and regulation of immune responses to HIV and HCV. Given that HCV may infect DCs[24, 75], and HCV proteins themselves may be able to interfere with DC functions[19, 58, 59], it is possible that impaired antigen presentation is the main reasons underlying the poor development and maintenance of immune responses to HCV. There are data to suggest that DC function in HCV-infected individuals is impaired[10], but this is controversial as it has not been confirmed in other studies[41]. HCV may induce defects in specific sets of DCs, such as plasmacytoid DCs, rather than cause a uniform defect[6]. Most studies infer DC function *in vivo* from the function of DCs derived from monocytes *in vitro*. Techniques now exist to isolate and study DCs directly *ex vivo*. Work from several groups that used blood-derived DCs have now shown impaired function in HCV[47, 75], although further studies are required.

HIV also interferes with DCs[20], probably with a different "profile" to HCV in terms of the subsets affected. This could partly explain the observed differences in immune response to these viruses[6]. HIV-induced impairment of DCs may not be reversed by HAART, even when HIV viral replication is suppressed[15], suggesting that this might be a further reason why HAART may have limited capacity to benefit HCV.

A recent study showed that HIV infection of monocytes may augment the capacity of HCV to replicate within them[38]. This suggests that HIV infection could potentiate a mild effect of HCV on antigen presentation. However, monocyte-derived DCs from co-infected persons have not shown additional impairment compared with HIV mono-infected persons[65].

A pervasive effect of HCV on $CD8^+$ T cell function?

Analysis of $CD8^+$ T cell responses in mono- and co-infection is important given their importance in antiviral immune responses[36, 81]. The $CD8^+$ memory T cell phenotype, for example the expression of the co-stimulatory molecules CD27 and CD28, varies for different viral infections[7]. This may occur because of accumulation of $CD8^+$ T cells at different stage of maturation. Thus in HCV infection, $CD8^+$ T cells are characteristic of a "central memory" (CM) phenotype, with expression of CCR7 and co-expression of both CD27 and CD28 ($CD27/CD28^{++}$). In contrast, CMV-specific $CD8^+$ T cells are mainly of an "effector memory" (EM) phenotype, with loss of CCR7, CD27, and CD28 ($CD27/CD28^{--}$), and acquisition of effector molecules such as perforin. It is not clear what

determines this pattern, but increased maturity of responses is not directly related to a greater burden of antigen, as viral replication with high levels of viremia occurs in HCV, whereas in latent CMV cellular expression of viral products continues only at low levels, if at all. It is therefore tempting to speculate that persistent viremia with HCV results from a failure to maintain appropriate numbers of effector T cells. This could occur through premature loss of EM cells, or defective maturation of CM cells (see Fig. 1).

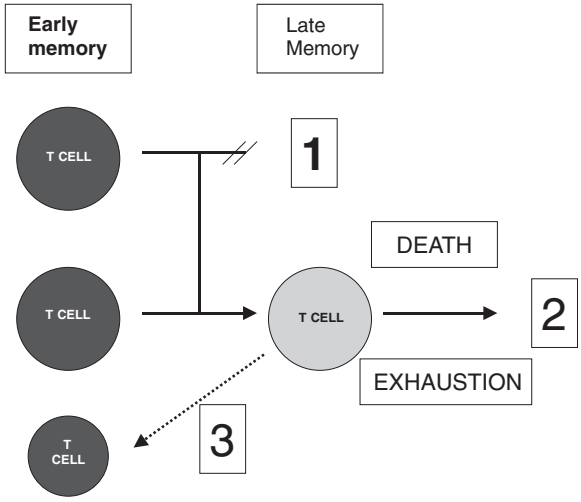

Figure 1. Possible reasons for presence of "stunted" HCV specific T cell responses. 1 – maturation may be impaired through, for example the presence of regulatory T cell populations; 2 – excessive death may occur because of migration to liver and apoptosis induced by infected hepatocytes. Exhaustion may occur because of an overwhelmed immune system, and finally 3 – stimulation of T cells *in vivo* may be impaired through generation of escape mutants. All three of these mechanisms may interplay. Many of the mechanisms involved may not be HCV-specific, and may therefore reduce the potency of HIV specific T cell responses resulting in impaired control of HIV viral infection.

We found that HCV infection not only affects the phenotype of HCV-specific T cells, but also has a pervasive influence on memory T cell responses to other pathogens[89]. CMV-specific T cells in HCV--infected persons show increased expression of CCR7, CD27, and CD28 and loss of typical markers of effector function such as perforin, compared with their expression in non-HCV-infected healthy controls. Theoretically, changes in such factors as cytokine levels (in tissues and in blood) may underlie this pervasive effect[78], given that cytokines such as interleukin (IL)-15, and the supernatant of CMV-infected cells[55] can alter T cell phenotype[4, 22].

This work provides an important clue as to how HCV may alter HIV progression: HIV-specific $CD8^+$ T cells are of an "intermediate phenotype", and HCV co-infection could skew this towards a CM and away from an EM phenotype. This could have consequences on the ability of HIV-specific T cells to suppress infection and lyse infected targets. In support of this hypothesis as a theoretical possibility is the finding that HIV-specific T cell responses tending towards an EM phenotype are associated with delayed progression of HIV[77].

An intra-individual comparison of $CD8^+$ T cell responses in co-infected subjects showed higher responses in terms of IFN-γ to HIV than to HCV[39], reflecting the situation in mono-infected individuals. However, this study did not include HIV mono-infected persons as controls, and a subtle functional effect may therefore have been missed.

It is also possible, however, that the T cell response to HCV is impaired through persistent exposure to high levels of antigen as opposed to being a cause of persistent viraemia. This could occur because of "escape" mutations of HCV or simple exhaustion of the immune system (see Fig. 1).

Does HCV enhance immune activation and progression of HIV?

Excessive immune activation is increasingly being seen as an important pathogenic link between HIV progression and AIDS[8, 27, 30]. Underlying this hypothesis is the observation that HIV infection results in huge and sustained increases in T cell turnover, which may result in eventual exhaustion of naïve $CD4^+$ and $CD8^+$ T cell reservoirs. The finding of lack of mature effector cells in HCV-infected individuals could readily be explained by excess turnover of this compartment with premature loss of EM T cells. This hypothesis is supported by the finding of elevated β-2 microglobulin levels, suggestive of high T cell turnover, in co-infected persons compared with HIV mono-infected controls[43]. The high incidence of auto-immune phenomena such as cryoglobulinemia and vasculitis occurring in hepatitis C also suggests immune dysregulation brought about by excessive immune activation. The HCV protein E2 on circulating virus complexes may directly mediate excessive immune activation as it lowers the threshold for T cell activation[80] following stimulation via the T cell receptor complex, and augments cytokine secretion, and proliferation (but not cytotoxicity).

Studies of T cell turnover in HCV compared with that seen in HIV in mono- and co-infection will be a key line of inquiry for future investigation.

Liver inflammation may modulate immune responses

Previously we proposed that the HCV-infected liver could provide a unique environment for T cells[85]. Exposure to infected hepatocytes may result in T cell death through apoptosis[31]. T cell recruitment to the HCV-infected-liver is not specific to T cells targeting HCV, as substantial recruitment of CMV-specific T cells to the liver is seen[82]. Data from an animal model of HIV (simian immunodeficiency virus, or SIV) have shown that SIV-specific T cells are concentrated in the liver[61]. With its large size and blood supply, and sinusoidal endothelium that is highly permeable to lymphocytes, the liver may serve as a "sink", and ultimately a "graveyard", for HIV-specific T cells. An immune system already over-activated by HIV may not be able to keep up with this additional drainage of its reserves.

Role of regulatory T cells in co-infection

Regulatory T cells are a subset of $CD4^+$ T cells that may modulate the immune system in both health and disease. An effect of HCV on regulatory T cells ($CD25^{hi}/CD4^+$ T cells) may underlie an effect of HCV in inducing immune tolerance. Recent data have suggested that the $CD4^+CD25^+$ subset is active in HCV-infected persons[67], and they have been shown to modulate $CD8^+$ T cell function in HIV mono-infected persons[1]. The non-specific action of T-regs marks them out as being of potential importance in co-infection, as an effect of HCV infection may be transferable to HIV-specific responses, and vice versa. Interestingly, persons with HCV-associated auto-immune vasculitis have lower levels of T-regs than HCV-infected persons without vasculitis[13], suggesting that T-regs may counter the deleterious consequences of chronic immune activation.

Effect of therapy with IFN-α and ribavirin on HIV progression

Therapy for HCV with IFN-α and ribavirin is only sustained for a period of 6 months to a year, in contrast to HAART, which is destined to be life-long. However, there is some evidence that it may be associated with reduced Th cell responses to CMV, HIV, and tuberculin[3, 40]. It is uncertain if this has any important sustained negative consequences. HIV viral load actually drops during IFN-α therapy[71], suggesting that IFN-α treatment may actually be beneficial for HIV.

DOES LATENT INFECTION WITH CMV WORSEN THE PROGNOSIS OF HIV?

Given that latent CMV will occur in more than 50% of HIV-infected persons, it is important to address if it is a co-factor for progression. This question has been debated for some time. Several studies have reported that CMV-seropositive persons show evidence of increased HIV viral replication, and more rapid progression to AIDS[56, 57, 83, 84]. But this has not been confirmed in other studies[11, 51, 74]. Several immunological mechanisms could, theoretically, allow CMV to be a co-factor for progression of HIV. In cases of CMV reactivation (which occurs in markedly immunosuppressed persons), even if this occurs at sub-clinical levels, there may be an additional stress on an already over-activated immune system. However, it has recently become clear that, in the era of HAART, a history of active CMV replication at presentation, as opposed to ongoing CMV replication *per se*, is an adverse prognostic factor for HIV, even when stratified for baseline $CD4^+$ T cell count and HIV viral load[18]. This raises the possibility that even with full suppression of CMV, on reconstitution of the immune system with HAART, CMV can result in adverse consequences for the immune system.

CMV has marked effects on the overall differentiation status of T cell pools. For example in children, CMV seropositivity was associated with an expansion of $CD45RA^+CD27^-$ cells (i.e. an EM phenotype)[37]. This was not seen with other common childhood infections (such as EBV, VZV, and MMR vaccine). In elderly subjects the influence of CMV is more marked, as it is associated with an inverted CD4/CD8 ratio and a high-risk immune phenotype, with increased risk of morbidity[49]. $CD8^+$ T cell responses to CMV increase with time, and in elderly persons may reach frequencies up to 30% of all $CD8^+$ T cells even for a single epitope. The memory responses may also be multi-specific[34] and therefore may place large demands on the immune system even in younger persons – when it could have telling effects on the capacity of the immune system to combat HIV.

A study in mice of heterologous immunity, that is the influence of a prior infection of a virus to influence the course of a second, different pathogen, show that protection may occur[33]. This study demonstrated protection to vaccinia virus following infection with lymphoid choriomeningitis virus (LCMV), a rapidly replicating RNA virus. However, unlike LCMV, CMV is a large β herpes virus, can establish latent infection, and has cumulative effects on the immune system over many years following acute infection. Therefore, theoretically at least, it could impair the emergence of HIV-specific immunity.

It is also possible that, like HCV, CMV has a pervasive effect on $CD8^+$ T cells regardless of their speci-

ficity, leading to increased T cell differentiation and maturity. As stated earlier, more mature $CD8^+$ T cell responses may be of benefit in HIV[77], but may lead to increased turnover, which could be detrimental, even if CMV infection is fully suppressed (see Fig. 2). Activation of T cells by CMV is likely to be a 2-edged sword in HIV infection: by promoting the generation of mature effector T cells it could in theory lead to better control of the virus (barring the emergence of virus escape mutants). However, if excess cell death/turnover is a major contributor to AIDS progression, the overall influence is likely to be negative. Although overtly unobtrusive, CMV may be a ghost-like ally of HIV infection in its assault on the immune system.

HIV AND GBV-C VIRUS

GBV-C was first described in 1995[32] and is found in around 1% of normal blood donors[62]. It seems that viremia can persist for years, but seroconversion coincides with viral clearance, as the presence of IgG antibody is very rarely seen in the many people who are GBV-C RNA^+. It is spread parenterally and by sexual transmission[52, 62]. Not surprisingly, it is commonly found in people with HIV infection. In a study of 138 HIV-infected persons[23], 36% had evidence of past infection (presence of IgG antibody). A higher proportion were PCR^+ compared with the number seen in healthy persons, suggesting that seroconversion may be delayed or prevented in persons who are HIV^+, possibly because of impaired immune responses. However, a cross-sectional analysis showed that status of infection was independent of current CD4 count at least[42].

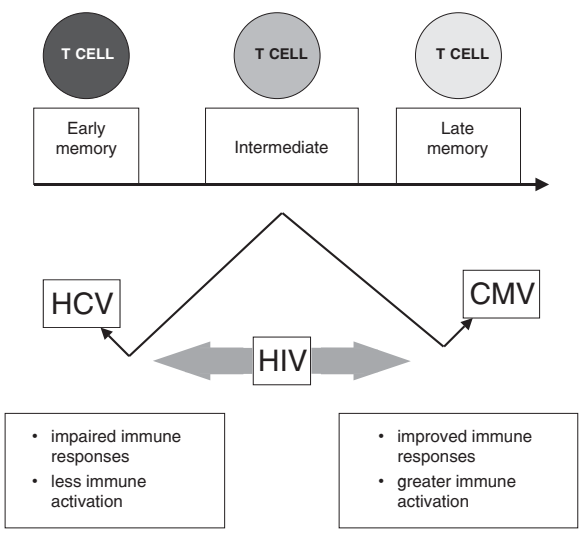

Figure 2. Diagram conceptualizing theoretical cross-influence in the generation of HIV specific immune responses in co-infection with HCV or CMV. If HCV is able to block maturation of T cells then this might benefit HIV by reducing immune activation, but direct control of HIV viral replication may be impaired. On the other hand if CMV infection is able to cause greater maturity of HIV specific T cells then this might lead to greater control of virus, but more deleterious immune activation.

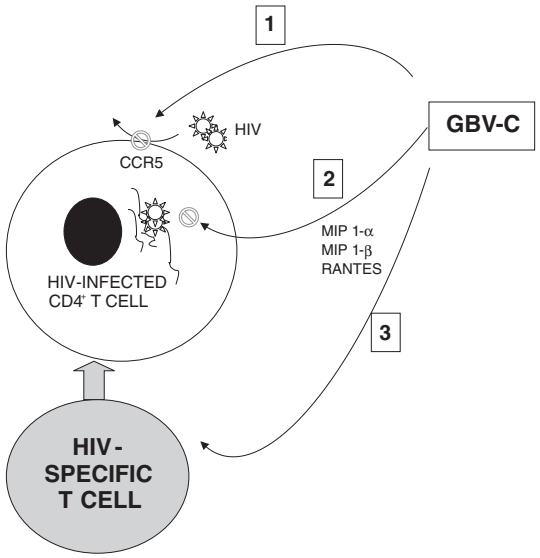

Figure 3. Possible mechanisms by which GBV-C might reduce HIV replication. 1 – blockade of entry through down-regulation of CCR5; 2 – inhibition of viral replication through eliciting production of RANTES and MIP 1-α and MIP 1-β; 3 – stimulation of type-1 immune responses to HIV.

The recent study by Williams et al.[86] showed that GBV-C viremia has a beneficial effect on HIV prognosis. This relationship does not appear to be confounded by immune status, as loss of GBV-V viremia, and therefore a successful immune response to GBV-C, was associated with a worse prognosis than in GBV-C uninfected persons. Persons with GBV-C RNA in serum also seem to have improved virological and immunological responses to HAART[54].

Three theories have been advanced to explain the interaction of HIV and GBV-C (see Fig. 3). Observations by Nunnari et al.[23, 51] suggest that GBV-C may favor anti-HIV responses by shifting to a Th1 cytokine profile through an unknown mechanism. A second hypothesis[48] is that GBV-C E2 protein can bind to CD81 on T cells. This in turn results in the release of RANTES (regulated-upon-activation, normal T cell expressed and secreted), which binds to and blocks the chemokine receptor CCR5. As CCR5 mediates HIV viral entry into T cells, through this process, GBV-C could act to inhibit HIV viral replication. CCR5 downregulation would explain the apparent protective role of GBV-C viremia, but would not explain the HIV rebound that leads to a worse

HIV prognosis when seroconversion finally occurs. It is also unclear why the hepatitis C protein HCV E2, which also binds CD81, does not also cause downregulation of CCR5[80]. HCV E2 can also stimulate lymphocytes. T cell activation by HGV E2 as occurs with HCV E2[80] could benefit HIV by stimulating immune responses, or could be deleterious by causing non-specific immune activation and a general increase in T cell turnover.

A recent study convincingly demonstrated the ability of GBV-C to suppress HIV viral replication *in vitro*[88]. Suppression of HIV replication was inhibited by antibodies to RANTES, MIP 1-α and MIP 1-β, and abolished completely when all these mediators were blocked. CCR5 was shown to be upregulated on activation of lymphocyes, and this was prevented by GBV-C, but the mechanism for this was not defined.

CONCLUSIONS

This review has aimed to give an overview of the emerging and important field of the immunology of viral co-infection. Our understanding of co-infection is generally hampered by a lack of insight into the strategies each virus may employ to evade the immune response and carve out its biological niche. In theory at least, these strategies could have the potential to antagonize or to synergize: disease from co-infection is likely to be more than the sum of its parts.

REFERENCES

1. Aandahl E. M., Moretto J., Hecht W. and Nixon D. F. (2004): Human $CD4^+CD25^+$ regulatory T-cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. J. Virol., 78, 2454–2459.
2. Agrati C, D'Offizi G., Narciso P., Selva C., Pucillo L. P., Ippolito G. and Poccia F. (2001): γδ T cell activation by chronic HIV infection may contribute to intrahepatic vdelta1 compartmentalization and hepatitis C virus disease progression independent of highly active antiretroviral therapy. AIDS Res. Hum. Retroviruses, 17, 1357–1363.
3. Alatrakchi N, Di Martino V., Thibault V., Benhamou Y., Katlama C., Poynard T., Autran B. (2004): Decreased frequencies of virus--specific T helper type 1 cells during interferon α plus ribavirin treatment in HIV-hepatitis C virus co-infection. AIDS, 18, 121–123.
4. Alves N. L., Hooibrink B., Arosa F. A. and van Lier R. A. (2003): IL-15 induced antigen-independent expansion and differentiation of human naive $CD8^+$ T-cells. Blood, 102, 2541–2546.
5. Andre P., Groettrup M., Klenerman P., de Giuli R., Booth B. L. Jr., Cerundolo V., Bonneville M., Jotereau F., Zinkernagel R. M. and Lotteau V. (1998): An inhibitor of HIV-1 protease modulates proteasome activity, antigen presentation, and T cell responses. Proct. Natl. Acad. Sci. USA, 95, 13120–13124.
6. Anthony D. D., Yonkers N. L., Post A. B., Asaad R., Heinzel F. P., Lederman M. M., Lehmann P. V. and Valdez H. (2004): Selective impairments in dendritic cell-associated function distinguish hepatitis C virus and HIV infection. J. Immunol., 172, 4907–4916.
7. Appay V., Dunbar P. R., Callan M., Klenerman P., Gillespie G. M., Papagno L., Ogg G. S., King A., Lechner F., Spina C. A., Little S., Havlir D. V., Richman D. D., Gruener N., Pape G., Waters A., Easterbrook P., Salio M., Cerundolo V., McMichael A. J. and Rowland-Jones S. L. (2002): Memory $CD8^+$ T-cells vary in differentiation phenotype in different persistent virus infections. Nat. Med., 8, 379–385.
8. Appay V. and Rowland-Jones S. L. (2002): Premature ageing of the immune system the cause of AIDS. Trends Immunol., 23, 580–585.
9. Asmuth D. M., Busch M. P., Laycock M. E., Mohr B. A., Kalish L. A., van der Horst C. M. and Viral Activation Transfusion Study (VATS) Group. (2004): Hepatitis B and C viral load changes following initiation of highly active antiretroviral therapy (HAART) in patients with advanced HIV infection. Antiviral Res., 63, 123–131.
10. Bain C., Zoulim F., Zarski J. P., Trepo C. and Inchauspe G. (2002): Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection. Gastroenterology, 120, 512–524.
11. Becherer P. R., Smiley M. L., Matthews T. J., Weinhold K. J., McMillan C. W. and White G. C. 2nd. (1990): Human immunodeficiency virus-1 disease progression in hemophiliacs. Am. J. Haematol., 34, 204–209.
12. Blackard J. T., Yang Y., Bordoni P., Sherman K. E., Chung R. T. and AIDS Clinical Trials Group 383 Study Team (2004): Hepatitis C virus (HCV) diversity in HIV-HCV-coinfected subjects initiating highly active antiretroviral therapy. J. Infect. Dis., 189, 1472–1481.
13. Boyer O., Saadoun D., Abriol J., Dodille M., Piette J. C., Cacoub P. and Klatzmann D. (2004): $CD4^+CD25^+$ regulatory T-cell deficiency in patients with hepatitis C-mixed cryoglobulinemia vasculitis. Blood, 103, 3428–3430.
14.. Cainelli F., Longhi M. S., Concia E. and Vento S. (2001): HIV-1 progression in hepatitis-C-infected drug users. Lancet, 357, 1361.
15. Carbonneil C., Donkova-Petrini V., Aouba A. and Weiss L. (2004): Defective dendritic cell function in HIV-infected patients receiving effective highly active antiretroviral therapy: neutralization of IL-10 production and depletion of $CD4^+CD25^+$ T cells restore high levels of HIV-specific $CD4^+$ T cell responses induced by dendritic cells generated in the presence of IFN-α. J. Immunol., 172, 7832–7840.
16. Daar E. S., Lynn H., Donfield S., Gomperts E., Hilgartner M. W., Hoots W. K., Chernoff D., Arkin S., Wong W. Y., Winkler C. A. and Hemophilia Growth and Development Study (2001): Relation between HIV-1 and hepatitis C viral load in patients with hemophilia. J. Acquir. Immune Defic. Syndr., 26, 466–472.
17. Day C. L., Seth N. P., Lucas M., Appel H., Gauthier L., Lauer G. M., Robbins G. K., Szczepiorkowski Z. M., Casson D. R., Chung R. T., Bell S., Harcourt G., Walker B. D., Klenerman P. and Wucherpfennig K. W. (2003): *Ex vivo* analysis of human memory CD4 T-cells specific for hepatitis C virus using MHC class II tetramers. J. Clin. Invest., 112, 831–842.
18. Deayton J. R., Sabin C. A., Johnson M. A., Emery V. C., Wilson P. and Griffiths P. D. (2004): Importance of cytomegalovirus viraemia in risk of disease progression and death in HIV-infected patients receiving highly active antiretroviral therapy. Lancet, 363, 2116–2121.
19. Dolganiuc A., Kodys K., Kopasz A., Marshall C., Do T., Romics L. Jr., Mandrekar P., Zapp M. and Szabo G. (2003): Hepatitis C virus core and non-structural protein 3 proteins induce pro- and anti-inflammatory cyokines and inhibit dendritic cell differentiation. J. Immunol., 170, 5615–5624.
20. Donaghy H., Stebbing J. and Patterson S. (2004): Antigen presentation and the role of dendritic cells in HIV. Curr. Opin. Infect. Dis., 17, 1–6.
21. Farci P., Shimoda A., Coiana A., Diaz G., Peddis G., Melpolder J. C., Strazzera A., Chien D. Y., Munoz S. J., Balestrieri A., Purcell R. H. and Alter H. J. (2000): The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. Science, 288, 339–344.

22. Geginat J., Lanzavecchia A. and Sallusto F. (2003): Proliferation and differentiation potential of human CD8$^+$ memory T-cell subsets in response to antigen or homeostatic cytokines. Blood, 101, 4260–4266.

23. Goubau P., Liu H. F., Goderniaux E. and Burtonboy G. (1999): Influence of CD4$^+$ lymphocyte counts on GB virus/hepatitis G virus carriership in HIV-positive individuals. J. Med. Virol., 57, 367–369.

24. Goutagny N., Fatmi A., De Ledinghen V., Penin F., Couzigou P., Inchauspe G. and Bain C. (2003): Evidence of viral replication in circulating dendritic cells during hepatitis C virus infection. J. Infect. Dis., 187, 1951–1958.

25. Grakoui A., Shoukry N. H., Woollard D. J., Han J. H., Hanson H. L., Ghrayeb J., Murthy K. K., Rice C. M. and Walker C. M. (2003): HCV persistence and immune evasion in the absence of memory T-cell help. Science, 302, 659–662.

26. Greub G., Ledergerber B., Battegay M., Grob P., Perrin L., Furrer H., Burgisser P., Erb P., Boggian K., Piffaretti J. C., Hirschel B., Janin P., Francioli P., Flepp M. and Telenti A. (2000): Clinical progression, survival and immune recovery during anti-retroviral therapy in patients with HIV-1 and hepatitis C virus co-infection. Lancet, 356, 1800–1805.

27. Grossman Z., Meier-Schellersheim M., Sousa A. E., Victorino R. M. and Paul W. E. (2002): CD4$^+$ T-cell depletion in HIV infection: are we closer to understanding the cause. Nat. Med., 8, 319–323.

28. Harcourt G., Hellier S., Bunce M., Satsangi J., Collier J., Chapman R., Phillips R. and Klenerman P. (2001): Effect of HLA class II genotype on T helper lymphocyte responses and viral control in hepatitis C virus infection. J. Viral Hepat., 8, 174–179.

29. Hayashi N., Kakimuma T., Soma Y., Grotendorst G. R., Tamaki K., Harada M. and Igarashi A. (2002): Connective tissue growth factor is directly related to liver fibrosis. Hepatogastroenterology 49, 133–135.

30. Hellerstein M. K., Hoh R. A., Hanley M. B., Cesar D., Lee D., Neese R. A. and McCune J. M. (2003): Subpopulations of long lived and short lived T-cells in advanced HIV-1 infection. J. Clin. Invest., 112, 956–999.

31. In C. (2003): Hepatic T cells and liver tolerance. Nat. Rev. Immunol., 3, 51–62.

32. Karayiannis P. and McGarvey M. J. (1995): The GB hepatitis viruses. J. Viral Hepat., 2, 221–226.

33. Karrer U., Oxenius A., Phillips R. and Klenerman P. (2001): Memory T cells: total recall or just a sense of deja vu? Nat. Immunol., 2, 991–993.

34. Khan N., Shariff N., Cobbold M., Bruton R., Ainsworth J. A., Sinclair A. J., Nayak L. and Moss P. A. (2002): Cytomegalovirus seropositivity drives the CD8 T-cell repertoire toward greater clonality in healthy elderly individuals. J. Immunol., 169, 1984–1992.

35. Kim A. Y., Lauer G. M., Ouchi K., Addo M. M., Lucas M., Schulze Zur Wiesch J., Timm J., Boczanowski M., Duncan J. E., Wurcel A. G., Casson D., Chung R. T., Draenert R., Klenerman P. and Walker B. D. (2004): The magnitude and breadth of hepatitis C virus specific CD8$^+$ T-cells depend on absolute CD4$^+$ T-cell count in co-infected individuals. Blood (in press).

36. Klenerman P., Wu Y. and Phillips R. (2002): HIV: current opinion in escapology. Curr. Opin. Microbiol., 5, 408–413.

37. Kuijpers T. W., Vossen M. T., Gent M. R., Davin J. C., Roos M. T., Wertheim-van Dillen P. M., Weel J. F., Baars P. A. and van Lier R. A. (2003): Frequencies of circulating cytolytic, CD45RA$^+$CD27$^-$, CD8$^+$ T lymphocytes depend on infection with CMV. J. Immunol., 170, 4342–4348.

38. Laskus T., Radkowski M., Jablonska J., Kibler K., Wilkinson J., Adair D. and Rakela J. (2004): Human immunodeficiency virus facilitates infection/replication of hepatitis C virus in native human macrophages. Blood, 103, 3854–3859.

39. Lauer G., Nguyen T. N., Day C. L., Robbibs G. K., Flynn T., McGowan K., Rosenberg E. S., Lucas M., Klenerman P., Chung R. T. and Walker B. D. (2002): Human immunodeficiency virus type-1-hepatitis C virus co-infection: intraindividual comparison of cellular immune responses against two persistent viruses. J. Virol., 76, 2817–2826.

40. Legrand E., Neau D., Galperine T., Trimoulet P., Moreau J. F., Pitard V., Lacut J. Y., Ragnaud J. M., Dupon M., Le Bail B., Bernard N., Schvoerer E., Houghton M., Fleury H. and Lafon M. E. (2002): CD4 T lymphocyte proliferative responses to hepatitis C virus (HCV) antigens in patients coinfected with HCV and human immunodeficiency virus who responded to anti-HCV treatment. J. Infect. Dis., 186, 302–311.

41. Longman R. S., Talal A. H., Jacobson I. M., Albert M. L. and Rice C. M. (2004): Presence of functional dendritic cells in patients chronically infected with hepatitis C virus. Blood, 103, 1026–1029.

42. Lopez Calvo S., Vela A., Castro A., Cid A., Aguilera A., Vega P., Hermida M., Regueiro B. J. and Pedreira J. D. (2003): GB virus C: lack of association with transaminases levels, CD4 and HIV viral load in aids patients. Ann. Medica Interna, 20, 175–178.

43. Macias J., Pineda J. A., Lozano F., Corzo J. E., Ramos A., Leon E., Garcia-Garcia J. A., Fernandez-Rivera J., Mira J. A. and Gomez-Mateos J. (2003): Impaired recovery of CD4$^+$ cell counts following highly active anti-retroviral therapy in drug naive patients coinfected with human immunodeficiency virus and hepatitis C virus. Eur. J. Clin. Microbiol. Infect. Dis., 22, 657–680.

44. Martin-Carbonero L., Benhamou Y., Puoti M., Berenguer J., Mallolas J., Quereda C., Arizcorreta A., Gonzalez A., Rockstroh J., Asensi V., Miralles P., Laguno M., Moreno L., Giron J. A., Vogel M., Garcia-Samaniego J., Nunez M., Romero M., Moreno S., de la Cruz J. J. and Soriano V. (2004): Incidence and predictors of severe liver disease in HIV virus infected patients with chronic hepatitis C: a European collaborative study. Clin. Infect. Dis., 38, 128–133.

45. Missale G., Bertoni R., Lamonaca V., Valli A., Massari M., Mori C., Rumi M. G., Houghton M., Fiaccadori F. and Ferrari C. (1996): 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., 98, 706–714.

46. Mohsen A. H., Easterbrook P., Taylor C. B. and Norris S. (2002): Hepatitis C and HIV-1 co-infection. Gut, 51, 601–608.

47. Murakami H., Akbar S. M., Matsui H., Horiike N. and Onji M. (2004): Decreased interferon-α production and impaired T helper 1 polarization by dendritic cells from patients with chronic hepatitis C. Clin. Exp. Immunol., 137, 559–565.

48. Nattermann J., Nischalke H. D., Kupfer B., Rockstroh J., Hess L., Sauerbruch T. and Spengler U. (2003): Regulation of CC chemokine receptor 5 in hepatitis G virus infection. AIDS, 17, 1457–1462.

49. Nilsson B. O., Ernerudh J., Johansson B., Evrin P. E., Lofgren S., Ferguson F. G. and Wikby A. (2003): Morbidity does not influence the T-cell immune risk phenotype in the elderly: findings in the Swedish NONA Immune Study using sample selection protocols. Mech. Ageing Dev., 124, 469–476.

50. Perez-Olmeda M., Soriano V., Asensi V., Morales D., Romero M., Ochoa A., Sanchez-Montero F., Santin M., Guardiola J., Blanch J., Nunez M., Jimenez-Nacher I., Garcia-Samaniego J. and The HCV/HIV Spanish Study Group (2003): Treatment of chronic hepatitis C in HIV-infected patients with interferon α-2b plus ribavirin. AIDS Res. Hum. Retroviruses, 19, 1083–1089.

51. Rabkin C. S., Hatzakis A., Griffiths P. D., Pillay D., Ragni M. V., Hilgartner M. W. and Goedert J. J. (1993): Cytomegalovirus infection and risk of AIDS in human immunodeficiency virus-infected hemophilia patients. National Cancer Institute Multicenter Hemophilia Cohort Study Group. J. Infect. Dis., 168, 1260–1263.

52. Ramia S., Mokhbat J., Sibai A., Klayme S., Naman R. and HIV Epidemiology Research Study Group (2004): Exposure rates to hepatitis C and G virus infections among HIV-infected patients: evidence of efficient transmission of HGV by the sexual route. Int. J. STD AIDS, 15, 463–436.

53. Rancinan C., Neau D., Saves M., Lawson-Ayayi S., Bonnet F., Mercie P., Dupon M., Couzigou P., Dabis F., Chene G. and Groupe d'Epidemiologie Clinique du SIDA en Aquitaine (GECSA) (2002): Is hepatitis C virus co-infection associated with survival in HIV-infected patients treated by combination antiretroviral therapy. AIDS, 16, 1357–1362.

54. Rodriguez B., Woolley I., Lederman M. M., Zdunek D., Hess G. and Valdez H. (2003): Effect of GB virus C coinfection on response to antiretroviral treatment in human immunodeficiency virus-infected patients. J. Infect. Dis., 187, 504–507.

55. Rowbottom A. W., Garland R. J., Lepper M. W., Kaneria S. S., Goulden N. J., Oakhill A. and Steward C. G. (2000): Functional

analysis of the $CD8^+CD57^+$ cell population in normal healthy individuals and matched unrelated T-cell depleted bone marrow transplant recipients. Br. J. Haematol., 110, 315–320.

56. Sabin C. A., Devereux H. L., Clewley G., Emery V. C., Phillips A. N., Loveday C., Lee C. A. and Griffiths P. D. (2000): Cytomegalovirus seropositivity and human immunodeficiency virus type 1 RNA levels in individuals with hemophilia. J. Infect. Dis., 181, 1800–1803.

57. Sabin C. A., Phillips A. N., Lee C. A., Janossy G., Emery V. and Griffiths P. D. (1995): The effect of CMV infection on progression of human immunodeficiency virus disease is a cohort of haemophilic men followed for up to 13 years from seroconversion. Epidemiol. Infect., 114, 361–372.

58. Sarobe P., Lasarte J. J., Casares N., Lopez-Diaz de Cerio A., Baixeras E., Labarga P., Garcia N., Borras-Cuesta F. and Prieto J. (2002): Abnormal Priming of $CD4^+$ T-cells bt dendritic cells expressing hepatitis C virus core and E1 proteins. J. Virol., 76, 5062–5070.

59. Sarobe P., Lasarte J. J., Zabaleta A., Arribillaga L., Arina A., Melero I., Borras-Cuesta F. and Prieto J. (2003): Hepatitis C virus structural proteins impair dendritic cell maturation and inhibit *in vivo* induction of cellular immune responses. J. Virol., 77, 10862–10871.

60. Saves M., Vandentorren S., Daucourt V., Marimoutou C., Dupon M., Couzigou P., Bernard N., Mercie P. and Dabis F. (1999): Severe hepatic cytolysis: incidence and risk factors in patients treated by antiretroviral combinations. Aquitaine Cohort, France, 1996–1998. Groupe d'Epidemiologie Clinique de Sida en Aquitaine (GECSA). AIDS, 13, 115–121.

61. Schmitz J. E., Veazey R. S., Kuroda M. J., Levy D. B., Seth A., Mansfield K. G., Nickerson C. E., Lifton M. A., Alvarez X., Lackner A. A. and Letvin N. L. (2001): Simian immunodeficiency virus (SIV)-specific cytotoxic T lymphocytes in gastrointestinal tissues of chronically SIV-infected rhesus monkeys. Blood, 98, 3757–3761.

62. Seifried C., Weber M., Bialleck H., Seifried E., Schrezenmeier H. and Roth W. K. (2004): High prevalence of GBV-C/HGV among relatives of GBV-C/HGV-positive blood donors in blood recipients and in patients with aplastic anemia. Transfusion, 44, 268–274.

63. Sheridan I., Pybus O. G., Holmes E. C. and Klenerman P. (2004): High-resolution phylogenetic analysis of hepatitis C virus adaptation and its relationship to disease progression. J. Virol., 78, 3447–3454.

64. Soriano V., Martin J. C. and Gonzalez-Lahoz J. (2001): HIV-1 progression in hepatitis-C infected drug users. Lancet, 357, 1361–1362.

65. Stebbing J., Patterson S., Portsmouth S., Thomas C., Glassman R., Wildfire A., Gotch F., Bower M., Nelson M. and Gazzard B. (2004): Studies on the allostimulatory function of dendritic cells from HCV-HIV-1 co-infected patients. Cell Res., 14, 251–256.

66. Sterling R. K., Wilson M. S., Sanyal A. J., Luketic V. A., Stravitz R. T., Contos M. J., Mills A. S. and Shiffman M. L. (2004): Impact of highly active antiretroviral therapy on the spectrum of liver disease in HCV-HIV coinfection. Clin. Gastroenterol. Hepatol., 2, 432–439.

67. Sugimoto K., Ikeda F., Stadanlick J., Nunes F. A., Alter H. J. and Chang K. M. (2003): Suppression of HCV-specific T cells without differential hierarchy demonstrated *ex vivo* in persistent HCV infection. Hepatology, 38, 1437–1448.

68. Sulkowski M. S., Moore R. D., Mehta S. H., Chaisson R. E. and Thomas D. L. (2002): Hepatitis C and progression of HIV disease. JAMA, 288, 199–206.

69. Sulkowski M. and Thomas D. L. (2003): Hepatitis C in the HIV-infected person. Ann. Intern. Med., 138, 197–207.

70. Sulkowski M. S., Thomas D., Chaisson R. E. and Moore R. D. (2000): Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. JAMA, 283, 74–80.

71. Talal A. H., Shata M. T., Markatou M., Dorante G., Chadburn A., Koch R., Neumann A. U., Ribeiro R. M. and Perelson A. S. (2004): Virus dynamics and immune responses during treatment in patients coinfected with hepatitis C and HIV. J. Acquir. Immune Defic. Syndr., 35, 103–113.

72. Tedaldi E. M., Baker R. K., Moorman A. C., Alzola C. F., Furhrer J., McCabe R. E., Wood K. C., Holmberg S. D. and HIV Outpatient Study (HOPS) Investigators (2003): Influence of coinfection with hepatitis C virus on morbidity and mortality due to human immunodeficiency virus infection in the era of highly active antiretroviral therapy. Clin. Infect. Dis., 36, 363–367.

73. Thursz M., Yallop R., Goldin R., Trepo C. and Thomas H. C. (1999): 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, 354, 2094–2095.

74. Touloumi G., Karafoulidou A., Gialeraki A., Katsarou O., Milona I., Kapsimali V., Mandalaki T. and Hatzakis A. (1998): Determinants of progression of HIV infection in a Greek hemophilia cohort followed for up to 16 years after seroconversion. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol., 19, 89–97.

75. Tsubouchi E., Akbar S. M., Horiike N. and Onji M. (2004): Infection and dysfunction of circulating blood dendritic cells and their subsets in chronic hepatitis C virus infection. J. Gastroenterol., 39, 754–762.

76. Valdez H., Anthony D., Farukhi F., Patki A., Salkowitz J., Heeger P., Peterson D. L., Post A. B., Asaad R. and Lederman M. M. (2000): Immune responses to hepatitis C and non-hepatitis C antigens in hepatitis C virus infected and HIV-1 co-infected patients. AIDS, 14, 2239–2246.

77. van Baarle D., Kostense S., van Oers M. H., Hamann D. and Miedema F. (2002): Failing immune control as a result of impaired $CD8^+$ T-cell maturation; CD27 might provide a clue. Trends Immunol., 23, 586–591.

78. van Lier R. A., ten Berge I. J. and Gamadia L. E. (2003): Human $CD8^+$ T-cell differentiation in response to viruses. Nat. Rev. Immunol., 3, 1–8.

79. Vlahakis S. R., Villasis-Keever A., Gomez T. S., Bren G. D. and Paya C. V. (2003): Human immunodeficiency virus-induced apoptosis of human hepatocytes via CXCR4. J. Infect. Dis. 188, 1455–1460.

80. Wack A., Soldaini E., Tseng C., Nuti S., Klimpel G. and Abrignani S. (2001): Binding of the hepatitis C virus envelope protein E2 to CD81 provides a co-stimulatory signal for human T cells. Eur. J. Immunol., 31, 166–175.

81. Walker C. M., Moody D. J., Stites D. P. and Levy J. A. (1986): $CD8^+$ lymphocytes can control HIV infection *in vitro* by suppressing viral replication. Science, 234, 1563–1566.

82. Ward S. M., Jonsson J. R., Sierro S., Clouston A. D., Lucas M., Vargas A. L., Powell E. E. and Klenerman P. (2004): Virus-specific $CD8^+$ T lymphocytes within the normal human liver. Eur. J. Immunol., 34, 1526–1531.

83. Webster A., Grundy J. E., Lee C. A., Emery V. C., Cook D. G., Kernoff P. B. and Griffiths P. D. (1989): Cytomegalovirus infection and progression towards AIDS in haemophiliacs with HIV infection. Lancet, 8, 63–66.

84. Webster A., Phillips A. N., Lee C. A., Janossy G., Kernoff P. B. and Griffiths P. D. (1992): Cytomegalovirus (CMV) infection, $CD4^+$ lymphocyte counts and the development of AIDS in HIV-1--infected haemophiliac patients. Clin. Exp. Immunol., 88, 6–9.

85. Willberg C. and Klenerman P. (2002): HCV-immunology: death and the maiden T-cell. Cell Death Different., 10, S39–47.

86. Williams C. F., Klinzman D., Yamashita T. E., Xiang J., Polgreen P. M., Rinaldo C., Liu C., Phair J., Margolick J. B., Zdunek D., Hess G. and Stapleton J. T. (2004): Persistent GB virus C infection and survival in HIV-infected men. N. Engl. J. Med., 350, 981–990.

87. Workalemahu G., Foerster M., Kroegel C. and Braun R. K. (2003): Human γ/δ T-lymphocytes express and synthesis connective tissue growth factor: effect of IL-15 and TGF-β1 and comparison with α/β T lymphocytes. J. Immunol. 170, 153–157.

88. Xiang J., George S. L., Wunschmann S., Chang Q., Klinzman D. and Stapleton J. T. (2004): Inhibition of HIV-1 replication by GB virus C infection through increases in RANTES, MIP-1α, MIP-1β, and SDF-1. Lancet, 363, 2040–2046.

Added in proof:

89. Lucas M., Vargas-Cuero A. L., Lauer G. M., Barnes E., Willberg C. B., Semmo N., Walker B. D., Phillips R. and Klenerman P. (2004): Pervasive influence of hepatitis C virus on the phenotype of antiviral $CD8^+$ T cells. J. Immunol., 172, 1744–1753.