

Impact of Human Immune Deficiency Virus Infection on Hepatitis C Virus Infection and Replication

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Abstract: Human immune deficiency virus (HIV) and human hepatitis C virus (HCV) infection are frequent in patients who have been exposed to blood or blood-derived products. It has been suggested that HIV infection increases HCV replication altering the course of HCV-related disease. However, it is not known if HIV directly enhances HCV replication or if its effect is the consequence of HIV infection of other cell types that control HCV replication (lymphocytes, macrophages). While the main cell targets for HIV infection are mononuclear leukocytes bearing CD4 and the chemokine receptors CCR5 and CXCR4, HCV was originally thought to be strictly hepatotropic, but it is now known that HCV can also replicate in peripheral blood mononuclear cells (PBMC). Therefore, in co-infected individuals, these two different viruses could share cell targets and interact either directly or indirectly. Some membrane receptors can be used by both HCV and HIV for entry into target cells, but the intracellular mechanisms shared by these viruses are not known. Lack of experimental systems providing suitable methods for the study of HCV replication in the presence or absence of HIV co-infection has hampered advances in this research area, but recent investigations are currently going on in order to answer these questions. This is an important issue, as knowledge of HIV/HCV interactions is required for the design of effective antiviral therapies.

INTRODUCTION

Human immune deficiency virus (HIV) and human hepatitis C virus (HCV) infection are frequent in patients who have been exposed to blood or blood-derived products. While the main cell targets for HIV infection are mononuclear leukocytes bearing CD4 and the chemokine receptors CCR5 and CXCR4, HCV was originally thought to be strictly hepatotropic. There is mounting evidence that HCV can also replicate in peripheral blood mononuclear cells (PBMC). Therefore, in co-infected individuals, these two different viruses could share cell targets and interact either directly or indirectly. It has been suggested that HIV infection could enhance HCV replication in co-infected patients [31, 144]. In HCV/HIV co-infected individuals (HCV/HIV), progression to hepatic fibrosis and liver cirrhosis have been observed [25, 47]. Likewise, progression to cirrhosis and terminal hepatic disease are increased during HCV/HIV co-infection [139]. The effectiveness of anti-HCV treatment is reduced in HCV/HIV [73], and plasmatic or hepatic viral load are higher in these patients than in those that had been infected with HCV alone [31, 144]. There is also evidence that acute HCV infection can raise HIV viremia in persons with otherwise well-controlled illness, and co-infection with HCV may accelerate progression from HIV infection to AIDS [47, 123]. The precise mechanisms that permit HCV and HIV to interact have not been well characterized, as a convenient experimental system for their study is not available.

In this review, we shall address the subject of *in vitro* HCV/HIV interaction, the steps of the immune process at

which these viruses are most likely to interact by sharing either membrane receptors, intracellular pathways or regulatory mechanisms, as well as the experimental systems that can be useful for future studies of their interplay. We will first focus on the role of dendritic cells (DC) in the initial steps of HCV and HIV infection, because there is mounting evidence that these cells have a key role in the assembly of an effective immune response against most pathogens. Moreover, DCs are known to possess receptors that can be used by HCV and HIV for the entry into the immune circuits and for infection. We will then examine the role of cytotoxic effectors in the control of HIV and HCV expansion. Finally, we will concentrate on the development of *in vitro* methods that may be useful to study HIV/HCV interaction at the cellular level.

Initial Steps of HIV and HCV Infection: DC as First Line Receptors of Viral Infection and as Key Players of the Immune Response to HIV and HCV

DC are nature's sentinels for initiating immune responses to invading pathogens and altered host cells. However, viral pathogens can use DC as vehicles for subverting the immune response and establishing infection in the host. Apparently, DC play an important role in HIV infection by sequestering the virus from its entry portal at mucosal sites and transferring it to lymphoid organs. HCV can also bind to C-lectin receptors (CLR) present in DC and these receptors are thought to open the way for DC infection and subsequent HCV replication in these cells [40, 81].

DC are the main antigen-presenting cells (APC) regulating the T cell response [8]. In the absence of external stimuli, DC remain in a resting state in which they have only a limited ability to prime naïve cells. However, in response to infection, DC undergo many phenotypic and functional changes which coordinately result in an improved ability to interact with T cells and promote T cell clonal expansion and differentiation. Immature DC, including Langerhans cells,

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splenic marginal zone DC and interstitial DC within lymphoid tissues, continuously sample self-antigen to maintain T-cell tolerance. Foreign antigens can also be grabbed by immature DC. If these cells are directly triggered by pathogens through their pattern-recognition receptors (like toll like receptors, TLR) [28], or indirectly induced through exposure to endogenous danger signals, such as material released from damaged cells or inflammatory mediators, they mature to immunogenic mature DC. Different TLR are used to recognize different classes of signals (derived from viruses, bacteria, damaged cells, etc); this is central to their role as sentinels of innate immunity and as translators of innate immunity into adaptive immunity [113]. It has been recently shown [50, 107, 137] that there is heterogeneity in the effector function of mature DC (licensed and unlicensed DC) according to the nature of the maturation stimulus. In some cases, interaction of pathogens with TLR does not lead to the generation of classic functional mature DC. It has been proposed that interaction of immature DC with HIV may lead to a semimature state whereby immature DC migrate to the lymph nodes but do not reach full maturation into mature DC. In this situation, semimature DC induce a state of tolerance rather than immunity to antigens [70]. DC constitute about 1% of peripheral blood mononuclear cells and can be divided into 2 major subpopulations that are phenotypically and functionally distinct: myeloid-derived dendritic cells (MDC) and plasmacytoid DCs (PDC) (Table 1). MDC are the most crucial APC involved in innate and adaptive host response to viral infections. Considerable work has been done using MDC obtained by *in vitro* stimulation of peripheral blood monocytes with growth stimulating factors. *In vitro*, under IL4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) growth conditions, CD14+ monocytes change to highly potent CD14- APC, termed monocyte-derived dendritic cells; CD34+ cells can also serve as *in vitro* precursors for MDC-like cells.

Table 1. Phenotype of MDC and PDC

	MDC	PDC
CD11c	+	-
CD123	-	+
CD83	+	-
DC-SIGN	++	++
CD4	+	++
CCR5	±	+
CXCR4	+	+
IFN- α (post viral challenge)	-	+++
IL12	++	-

The second subpopulation of migrating blood DC, termed PDC are distinguished from MDC because they do not express CD11c, they express high levels of CD123 (IL3 receptor) and produce high amounts of the antiviral protein IFN- α , in response to HIV and other virus infections [18, 29, 100]. Thus, PDC are important in innate immunity to viral infections. Unlike MDC, which migrate to tissues and intercept invading pathogens before their migration to the lymph

nodes, PDC migrate directly from blood to the secondary lymphoid tissue. Differentiation of blood monocytes in the presence of IFN- α and GM-CSF leads to DC expressing TLR-7 that exhibit many of the functional characteristics of PDC [93], indicating that DC precursors can expand and differentiate into cells of diverse phenotype and function influenced by the composition of the surrounding milieu and the nature of the differentiating stimulus.

HIV Binding and Infection of DCs

Migrating DC are probably responsible for transport of HIV to T cell regions of draining iliac or colonic lymph nodes. Infection of an activated CD4 lymphocyte is more efficient when the virus is transferred during a DC-T cell response (Fig. 1). During the early phase of HIV replication *in vivo*, this DC-T lymphocyte synergy could play a major role in initial viral replication [156].

Although with lower frequency than macrophages and activated CD4+ T lymphocytes, both DC types (MDC and PDC) could be infected by HIV-1 *in vitro* and *in vivo* [86]. However, virus replication was more efficient in PDC. Isolation of highly purified MDC and PDC obtained from HIV-1 infected patients, demonstrated that both subpopulations were infected with HIV and that some MDC contained integrated provirus [86]. Also, both populations were severely impaired in their ability to stimulate T-lymphocyte proliferation. Loss of circulating MDC and PDC is likely to be an important factor in the decline of acquired and innate response in HIV-1 infection.

In addition, thymic DC subsets can be infected by HIV, sustaining high levels of HIV replication. HIV-infection of thymic DC had cytopathogenic effects leading to DC death in accordance to the level of viral replication. Thus, in addition to cooperate in the expansion of HIV infection in the thymus, DC infection by HIV could affect thymopoiesis in infected individuals [126].

The C-type lectin DC-SIGN (CD209), present in immature DC, was initially suggested to play a key role in HIV dissemination by DC *in vivo* [41, 147]. However on certain DC subsets identified *in vivo*, other C-type lectin receptors (CLR) such as langerin, on Langerhans cells (LCs), as well as mannose receptors (MR), on dermal DC, must be more relevant [147]. Therefore, DC-SIGN although important, may represent only one of several CLR present on DC that are able to interact with HIV *in vivo* [115, 148]. It has been postulated that DCs are just cellular viral "Trojan horses", which transfer virus to permissive cell types *via* DC-SIGN binding without being infected themselves. Several authors [71, 89, 97] have shown that DC-SIGN binding results in HIV internalization in DC-SIGN-transfected cells. However, recently it was suggested that DC-SIGN-mediated internalization was dispensable for enhancement of HIV-1 transfer from DC to T cells. Apparently, DC-SIGN would facilitate DC infection, with subsequent transfer of newly synthesized, DC-derived viral particles to T cells [19]. Direct transfer of HIV from the endosomal pathway of DC to CD4 lymphocytes is unlikely to be selective for CXCR4 or CCR5 HIV co-receptors, whereas "*de novo*" infection of DC is likely to select for CCR5 tropic virus.

Recently, expression of a DC-SIGN homolog was demonstrated on liver sinusoidal cells and placenta capillary

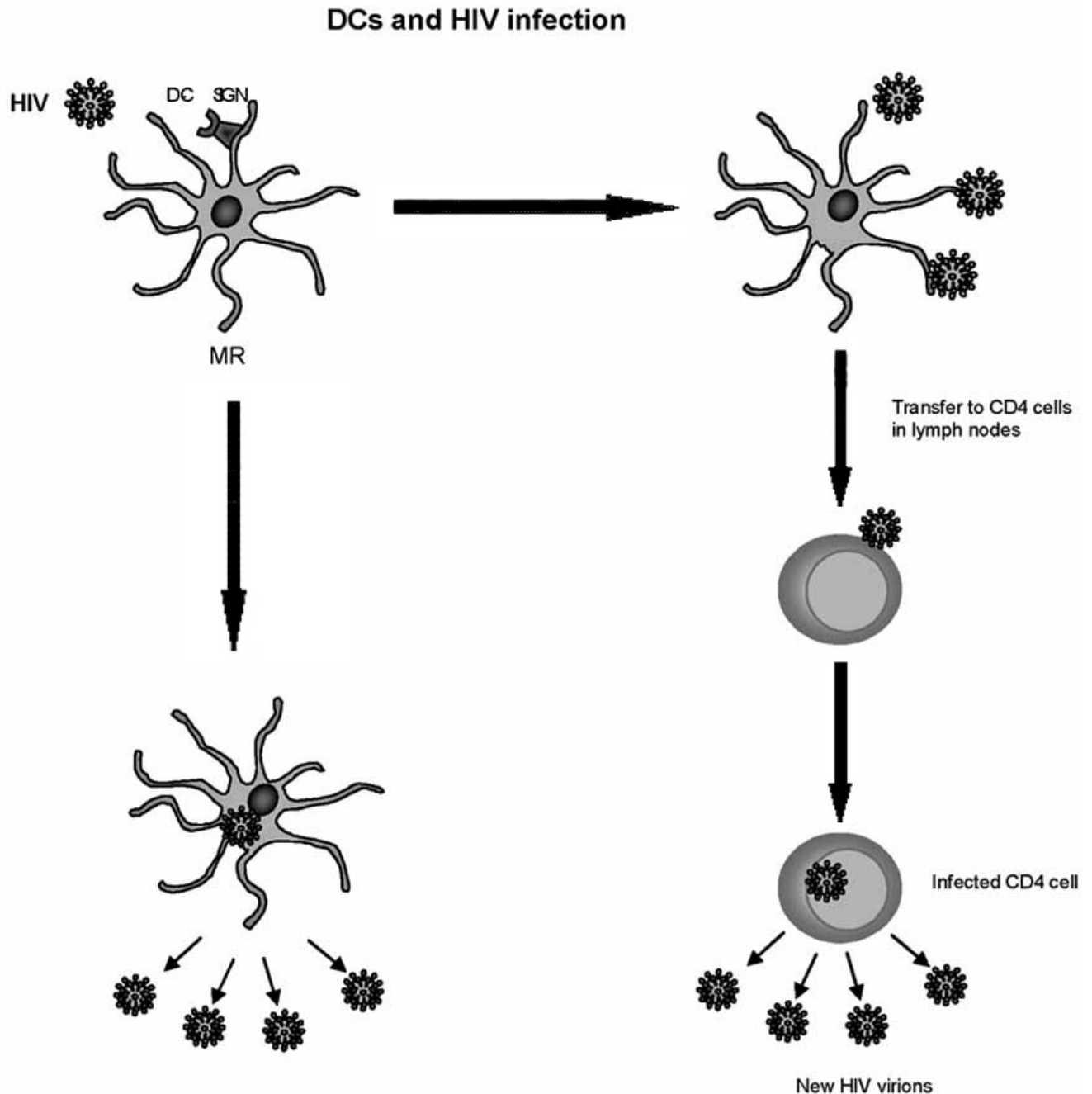


Fig. (1). Differentiation of dendritic cells.

Precursor dendritic cells (DC) become immature DC with low (lo) expression of CD80/CD86 markers, major class II histocompatibility antigens (MHC II) and CD40. Immature DC can induce tolerance of CD4 T cells to antigen or become mature DC. Exposure to inflammatory cytokines alone leads to development of unlicensed mature DC, unable to induce differentiation of CD4 T lymphocytes into effector cells. Interaction with pathogens bearing toll like receptor (TLR) ligands leads to proliferation and differentiation of CD4 T lymphocytes into effector cells.

endothelium, but not on DC. This DC-SIGN related molecule (DC-SIGNR) has also the capacity of binding HIV gp120 and is co-expressed with DC-SIGN at low levels on lymph node sinus endothelium. Therefore, it could also contribute to HIV transfer at this site [12, 105, 106].

Loss of PDC as a Result of HIV Infection

Loss of DC alone would lead to a decline in T-lymphocyte responses through impaired antigen presenta-

tion. In addition, specific loss of DC involved in the release of mediators (inflammatory cytokines, interferon) that are important for the balance of the immune response would further affect the ability of the HIV infected host to control opportunistic infections and maintain an intact immune defense system [32]. It has been proposed that MDC and PDC play opposing roles in HIV infection of T cells. While MDC appear to facilitate HIV infection through capture of the virus and transmission to T cells, PDC would inhibit HIV

replication in T cells through the secretion of IFN- α and other small molecules [48].

The absolute number of circulating PDC (> 2 cells/ μ l) can be used to monitor the immune system in HIV-infected patients. The number of PDC (INF- α producing cells) in the blood is markedly reduced in AIDS patients but increased in asymptomatic HIV+ long term survivors [100]. The PDC number may decline to low levels and then remain relatively constant even when the CD4 levels continue to drop [136]. The concept that both PDC and CD4+ T lymphocytes can be independently impaired after HIV infection was corroborated by the study that compared the production of INF- α (which is mainly PDC dependent) versus CD4 levels in a large cohort of HIV-infected patients [132]. These deficiencies were found not only in terms of absolute numbers of DC/ μ l of blood but also in terms of their relative number, indicating that there was a selective loss of these subpopulations that cannot be attributed to generalized leucopenia. A possible explanation for the loss of PDC in HIV-infected patients is that they could have left the peripheral blood to enter into the secondary lymphoid organs or tissues. However, the plasma viral load of HIV was found to significantly correlate with the levels of functional IFN- α - producing cells (as measured by ELISPOT), rather than with the absolute numbers of PDC. Susceptibility to opportunistic infection and disease progression did not occur unless both the IFN- α production and the CD4+ T cell level were critically compromised [132]. Table 2 summarizes the consequences of HIV infection on DC balance and function.

Table 2. Consequences of HIV Infection on DC Function

• Massive loss of PDC
• Reduced IFN- α/β production
• Reduced number of MDC
• Impaired Th1 and Th2 responses

HCV Binding and Infection of DC

A remarkable feature of HCV infection and the ensuing HCV-associated disease is the ability of this virus to persist in the majority of infected individuals [30]. Anti-HCV therapy (recombinant IFN- α in conjunction with the nucleoside analog ribavirin) is not fully adequate and only temporarily effective in most cases [43, 52]. HCV is a small, enveloped, plus-strand RNA virus belonging to the *Flaviviridae* family. Its genome is a single strand RNA 9600 nucleotides in length, encoding a single polyprotein that is processed into structural proteins located at the N-terminus (core, E1, E2 and p7) and 6 non structural proteins [11, 46, 51]. The identity of the receptor for HCV remains elusive. The low density lipoprotein (LDL) receptor (LDLR) has been shown to mediate internalization of HCV particles associated to LDL [35]. The tetraspanin CD81 has been identified as a high affinity binding receptor for recombinant E2 from HCV genotype 1a [157]. However, since LDLR and CD81 are expressed in most cell types, the hepatic tropism of this virus cannot be ascribed to the presence of these membrane molecules [163]. Viral infection is characterized by a high rate of chronicity that in 20% of the cases leads to liver cirrhosis, with the eventual development of hepatocellular carcinoma

[128]. This virus is highly variable and exists in infected persons as a quasispecies that consists of a pool of related but genetically distinct variants [133]. Replication of the HCV genome has been demonstrated in primary hepatocytes and hepatocyte cell lines [53] as well as in hematopoietic cells [9, 10, 111]. Since the genomic sequence of HCV was determined, progress has been made towards understanding the function of the HCV encoded proteins, although lack of an efficient *in vitro* replication system or a small animal model for experimental infection has hampered unraveling the complex nature of HCV/host interaction. The report of HCV-RNA in PBMC suggests that separate HCV reservoirs may coexist and that HCV may use different receptors to infect different cells [75]. Recently, the role of CLR as receptors of HCV has been raised [81]. Thus DC-SIGN and a related CLR molecule present in the endothelium lining hepatic sinusoids and not in DC (L-SIGN) were shown to bind HCV envelope glycoprotein E2 through high mannose glycans. It is tempting to speculate that subsequent to binding to endothelial L-SIGN [40], HCV could be transmitted to the surrounding hepatocytes in a “trans” interaction as proposed for HIV bound to DC-SIGN (Fig. 2). In addition, efficient binding of soluble E2 to DC-SIGN and DC-SIGNR has also been demonstrated in cell lines and human primary endothelial cells [106]. Both mature and immature-MDC could bind soluble E2, but while DC-SIGN was required for E2 capture by immature MDC, binding of E2 to mature MDC was partly independent of DC-SIGN expression, suggesting that other surface molecules may mediate HCV glycoprotein interactions in these cells [106].

There is evidence that, in addition to binding to DC receptors, HCV can replicate in DC obtained from patients chronically infected with HCV [6, 44, 103] suggesting that MDC may constitute a reservoir in which HCV replication takes place during natural HCV infection. Furthermore, as demonstrated for HIV infection, both MDC and PDC were depleted and their function was impaired in HCV infected patients with chronic hepatitis [61], which may explain their poor antiviral adaptive immune response. There is controversy on the function of both PDC and MDC in HCV-infected patients. While in some studies it was reported as normal [3, 104] in other studies impaired IFN- α secretion was observed in HCV patients receiving therapy (recombinant IFN- α and ribavirin) and this finding was related to the number of PDC identified in the blood, suggesting that PDC depletion and decreased IFN- α secretion were the consequence of treatment and not the result of viral infection *per se* [44]. In other studies, functional defects of DC (impaired allostimulatory function, increased IL-10 production, reduced expression of TLR2) were observed in individuals with chronic HCV infection [6, 59-62]. In any case, functional defects of peripheral blood DC (mainly those of the PDC subset) could dramatically impair innate and adaptive anti-HCV immune response leading to viral persistence [42].

Cell-Mediated Immune Response to HIV and HCV

Impaired immune response to HIV and the generalized immunodeficiency that characterizes HIV disease can be explained at least in part, because the main HIV targets are CD4 T lymphocytes and other CD4 bearing hematopoietic cells that are necessary for a correct balance of the immune

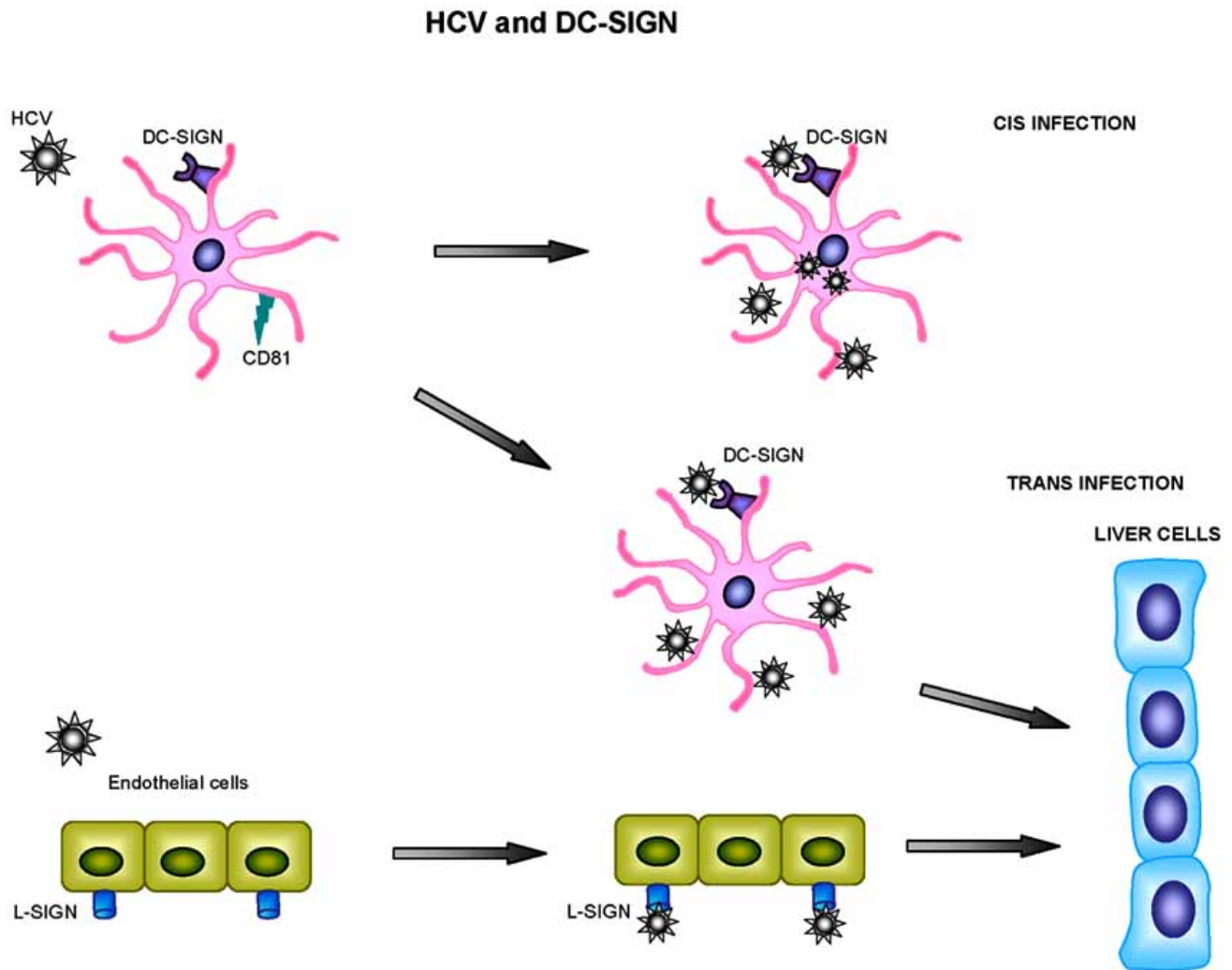


Fig. (2). Immune response related factors determining HIV or HCV persistence.

system. In addition to HIV-induced cell death, infection may alter the function of CD4+ cells [131].

On the contrary, the mechanisms whereby HCV establishes a persistent infection remain elusive [21]. Subversion of host immune responses by HCV through different evasion pathways could underlie the high rate of occurrence of chronic infection [38]. The strategies of evasion of the immune response followed by HCV are designed to bypass or ignore both the innate and the adaptive immune response [155]. In this regard, both its high rate of mutation and its ability to resist the defense machinery put forward by the adaptive immune response, help to establish chronic infection [138]. In order to prevent the occurrence of persistent HCV infection and its associated liver diseases, it will be important to establish if failure to produce an efficient immune response to HCV stems from the impact of antigen overload during immunological priming [28], to defects of antigen presentation [158], to hyperinduction of regulatory T cells [124], or to other unknown reasons [155].

CD8+ T Cells and HIV Infection

The importance of CD8+ T lymphocytes in containing HIV replication was appreciated soon after the onset of the

AIDS epidemic. CD8+ T lymphocytes were shown to inhibit HIV replication *in vitro* in autologous lymphocytes [153]. The importance of CD8+ T lymphocytes was then demonstrated *in vivo* showing the expansion of CD8+ cytotoxic T lymphocytes (CTL) with oligoclonal V β repertoires in acutely infected patients at a time when neutralizing-antibody was absent and viremia started to decline [69]. Virus specific CTL responses have also been implicated in chronic HIV infection, in particular in patients with slow declines of CD4+ T lymphocytes, low HIV viral load in plasma and stable clinical condition [96] and in seronegative Gambian prostitutes who remained uninfected by HIV in spite of frequent exposure [118]. A significant inverse correlation was reported between the frequency of peripheral blood HIV specific CTL and plasma viral load, supporting the importance of the role of CTL in controlling HIV replication [98]. Concerning CD4+ T lymphocytes, it is possible that CD4+ virus specific T cells provide the immunological help necessary for expanding and maintaining HIV-specific CD8+ CTL [2]. In many studies, the ability of CD8+ T cells to respond to HIV antigens presented in the correct HLA context is measured in terms of their ability to synthesize IFN γ . However, IFN γ synthesis is not equivalent to the capacity of these cells to kill HIV-infected cells, and the num-

ber of virus specific CD8+ T cells generally surpasses the number of infected cells replicating the virus [78]. Long term non progressor and progressor HIV-infected individuals had similar numbers of CD8+ HIV-specific T cells capable of synthesizing IFN γ . However, in addition to their frequency, it is important to consider breadth of epitope recognition and functional quality of HIV-specific CD8+ effector cells. In this regard it has been reported that HIV non progressors maintain highly functional HIV-specific CD8+ T cells [15].

Impaired CD8+ T cell maturation could be the cause of the CD8+ T cell defect in HIV-infected patients. Multiple factors may be involved in the development of incomplete or inefficient maturation, among them defective DC presentation, antigen initial concentration and persistence, chronic activation, the cytokine milieu, activation by CD70 expressed on B lymphocytes, cytokine receptor deficiencies, etc [5, 87, 91, 99]. As a result of these multiple factors, defense mechanisms would fail allowing HIV persistence as has been proposed in other animal models [162].

CD8+ T Cells and HCV Infection

The development of methods useful to identify virus specific effector CD8+ T cells [72] has helped to establish the role of these cells in resolution of HCV-associated disease. Using one of the methods, the ELISPOT assay that involves the detection of cells secreting IFN γ , the number of HCV-specific CD8+ T cells during the first 6 months after onset of disease appeared to be associated to eradication of HCV infection [49]. However, chronic HCV infection can be established in the presence of persisting virus-specific CD8+ responses [36]. While circulating memory CD8+ T lymphocytes display the early/intermediate effector phenotype in chronic HCV+ patients [4], they may possibly be hampered in generating efficient effector cells *in vivo* [36]. Interestingly, it has been shown that while HCV-specific CD8+ T cells have a T central memory (T_{CM})-like profile displaying surface receptors characteristic of the early/intermediate phenotype (CD27, CD28 and CCR7) [4], they do not express the homing molecule CD62L as most T_{CM} [82] (Table 3). This affects not only the quality of HCV-specific CD8+ T cell responses, but also the quality of CD8+ effector T cells against other virus, such as cytomegalovirus (CMV), suggesting that HCV-infection may induce a generalized defect in the maturation of effector CD8+ T cells [82]. The frequency of CD8+ T cells recognizing HCV antigens was higher in chronic HCV than in patients who recovered from infection, but these cells were functionally impaired, as they did not proliferate or produce IFN γ and they failed to kill HCV-infected targets [154]. While the information on CD8+ T cell responses points to their protective role in HCV infection, CD4+ T cell responses are also important. Thus, the CD4+ T cell response against HCV persisted in acutely infected patients who cleared HCV infection, while it disappeared in those who recurred after initial control of HCV infection [42]. Preserved CD4 response to HCV would be necessary to maintain CD8+ T effector cell function during the chronic phase, as shown for other viral infections [162]. Some of the defects of DC function described before [59, 61] or their inability to secrete IL-15 [57] could be related to failure to develop an efficient CD8+ T cell response during the chronic phase.

Table 3. Role of CD8+ T Lymphocytes in Control of HIV and HCV Infection

A.- CD8 T cells and HIV control	
•	Increased cytotoxic (CTL) responses against HIV in patients with better evolution [96].
•	Increased CTL responses in HIV resistant Gambian prostitutes [118].
•	Inverse relation between CTL and HIV viral load [98].
•	Defects in CD8 maturation in HIV progressor patients [4, 91].
B.- Failure of CD8 T cells in HCV control	
•	Altered CD62L expression in CD8+ cells from HCV patient [82].
•	Lack of HCV-induced proliferation and IFN γ release or cytolysis of HCV+ targets in HCV patients [154].
•	Lack of CD8+ T cell help to induce CD8+ T effector cells in HCV patients [42].

HIV/HCV Co-Infection and the Immune Response to Each Virus

Since HIV and HCV share common routes of infection, patients co-infected with both viruses are frequently found and there is evidence that the course of virus-associated disease is different (at least for HCV) in co-infected patients than in singly infected patients. There is evidence that HIV influences the course of HCV infection in several ways (Table 4).

Table 4. HIV/HCV Co-Infection and Immune Response to HCV

•	Decreased time to progress to HCV-related liver disease in HIV/HCV co-infected than in HCV patients [76].
•	Accelerated progression to cirrhosis or liver carcinoma in HIV/HCV co-infected than in HCV patients [14, 39, 83].
•	Higher death rate in HIV/HCV co-infected than in HCV patients [26, 77].

First, HCV-associated disease progresses more rapidly in HIV/HCV co-infected individuals. In general HCV-related liver disease takes about 30 years in a mono-infected individual [76] but is significantly shorter in HIV/HCV infected patients [14, 83]. The cumulative number of deaths due to failure of the liver after the first exposure to HCV was four times greater in HIV/HCV individuals than in singly infected HCV patients [26]. These results were confirmed in recent studies showing that the death rate associated to hepatic disease was 7 times higher in HIV/HCV than in HCV patients, and these figures were even higher in AIDS patients [77]. The lapse required for the development of hepatocellular carcinoma was shorter in HIV/HCV patients [39]. Likewise, 25% of the patients in the HIV/HCV group developed cirrhosis after 15 years of HCV infection, while only 6.5% of singly infected HCV+ patients do it in the same period [125]. While the grade of hepatitis was associated to HIV stage and the consequent functional defect in the immune system, cirrhosis was initiated during the initial stages of HIV infection, when the function of the immune was relatively preserved [27]. There are several proposals to explain why HIV infection accelerates the course of HCV-associated disease.

In general it is attributed to the generalized state of immunosuppression caused by HIV-infection [108, 116], but enhanced fibrogenesis [130] or increased liver infiltration by CD3+CD56+ cells (a subset of natural killer cells) [120] could also favor liver damage.

Second, HIV infection results in increased HCV viral loads [31, 88, 143]. Loss of immune control of HCV infection in HIV+ individuals, may underlie the observed increments of the HCV viral load [13, 24, 31, 88, 135, 145]. On the other hand, other authors failed to observe this inverse association between failure of the immune system and increased plasma HCV RNA [23, 114, 129, 143, 160]. How HIV infection results in increased HCV replication is not clear. Beld *et al.* [13] observed that independently of the immune suppression induced by HIV (reflected by decreased numbers of CD4 lymphocytes) HIV infection had an influence in the levels of HCV RNA in plasma. A role for the HIV regulator gene *tat* has been proposed [151] since a putative *tat*-binding motive in the NS4 HCV protein could be a target for the regulatory action of HIV *tat* [33].

Third, the response to anti HCV treatment is lower in HCV/HIV patients than in HCV singly infected individuals. In mono-infected patients, responses to pegylated interferon and ribavirin can reach 50-80% [37, 84], while in those that are HIV co-infected, treatment is effective only in 20-35% [73, 102]. Co-infected individuals would require higher doses of anti HCV treatment to achieve similar results to HCV singly infected individuals [94, 134]. In agreement with this, the chance of reaching spontaneous clearance of HCV infection are reduced from 10-15% in the HCV group to 2.5% in the HIV/HCV group [1, 24, 142, 150]. Clearance has been associated to a strong and sustained CD4 response that is necessary to induce an effective CD8 response against HCV. In addition to the effects of HIV infection *per se* on HCV-disease evolution, one must consider the impact of highly active antiretroviral therapy (HAART). It has been shown that initiation of HAART can have an effect on HCV viral load. HCV viral load was shown to increase [109, 112, 122] in some cases with temporary increase in the hepatic transaminases [22, 117, 122, 149]. However, HAART treatment has a beneficial long term effect on HCV-associated liver disease [85, 110, 146]. The effects of HAART can be indirect, through their influence on the immune balance or on the recovery of cells that can be targets for HCV infection [34, 90, 101].

Data regarding the effects of HCV infection on HIV disease progression are not clear. Initial reports suggested that HCV infection accelerated progression to AIDS [47, 123], but these results were not confirmed by further studies [140]. In spite of this, there is evidence that HCV infection can generally affect the function of cells that are central for the assembly of an efficient immune response against HIV and other viruses [45, 82]. Therefore, both viruses could mutually affect the mechanisms that control their persistence and expansion.

Experimental Systems for the Study of HCV/HIV Interaction

In vitro systems for the study of HIV replication, cell cycle and molecular biology are widely used [68]. Unfortunately, the absence of an efficient culture model that could

reproduce virus life cycle has severely limited the analysis of many aspects of HCV infection. Primary hepatocyte cultures and hepatocyte cell lines have been used to study HCV infection *in vitro* [56, 63, 121]. However, reproducibility of the results and HCV replication levels were low. On the other hand, HCV infection of different target cells using HCV+ serum did not provide satisfactory results due to the variability of serum-derived virus infectivity [11]. In 1999, a culture system that is suitable for the study of various aspects of HCV RNA replication and persistence became available and its advantages have been reported [80]. The "replicon system", an experimental model that allows efficient propagation of HCV replicons in Huh-7 (a human hepatoma cell line) has been useful for the study of HCV. Replicons are subgenomic or genomic units of HCV obtained on the basis of consensus genomes cloned from the liver of HCV infected patients [64, 80]. The recent establishment of a robust cell culture model that produces infectious HCV particles provides a powerful tool for the analysis of host-virus interactions and the screening of antiviral compounds [21, 79, 164]. Based on this model, different systems of HCV replication are currently being applied to elucidate many aspects of the virus-host interactions including viral entry, assembly and release that were previously unapproachable [17, 55, 58, 92, 159].

Regarding animal models, infection of chimpanzees provides means to study HCV infection, albeit at a high cost and with the limitations of the experimental use of species that may be approaching extinction [11]. An alternative is the use of transgenic mice expressing HCV proteins [65, 67], or experimental infection obtained by transplant HCV infected liver fragments into lethally irradiated mice reconstituted with bone marrow from immunodeficient mice (SCID mice) [54]. While these models have provided adequate means for the study of direct antiviral effects or gene therapy, they can not be used for the analysis of the immune response to HCV.

In order to solve the issue of HCV/HIV interaction it is necessary to obtain experimental models where the influence of one virus on the other can be studied at the cellular level in a system that allows the analysis of a few variables at a time.

Using *in vitro* infection systems, Laskus and collaborators have demonstrated that HIV could facilitate replication of HCV in native human macrophages either by rendering cells more susceptible to HCV infection or by increasing HCV replication. Although the mechanisms by which HIV facilitates HCV infection are still unclear, a positive correlation between HIV viral load and levels of HCV replication in macrophage cultures has been observed in the early stage of infection [74].

Some reports based on the addition of viral proteins to hepatocyte lines or primary hepatocyte cultures revealed that the two viruses had synergistic effects on the regulation genes of the infected cells. *In vitro* models demonstrated viral interactions showing that HCV and HIV structural proteins might injure uninfected hepatocytes by an "innocent bystander" mechanism. The authors could demonstrate that signaling pathways could be involved in hepatic injury in co-infected patient cells [7, 95].

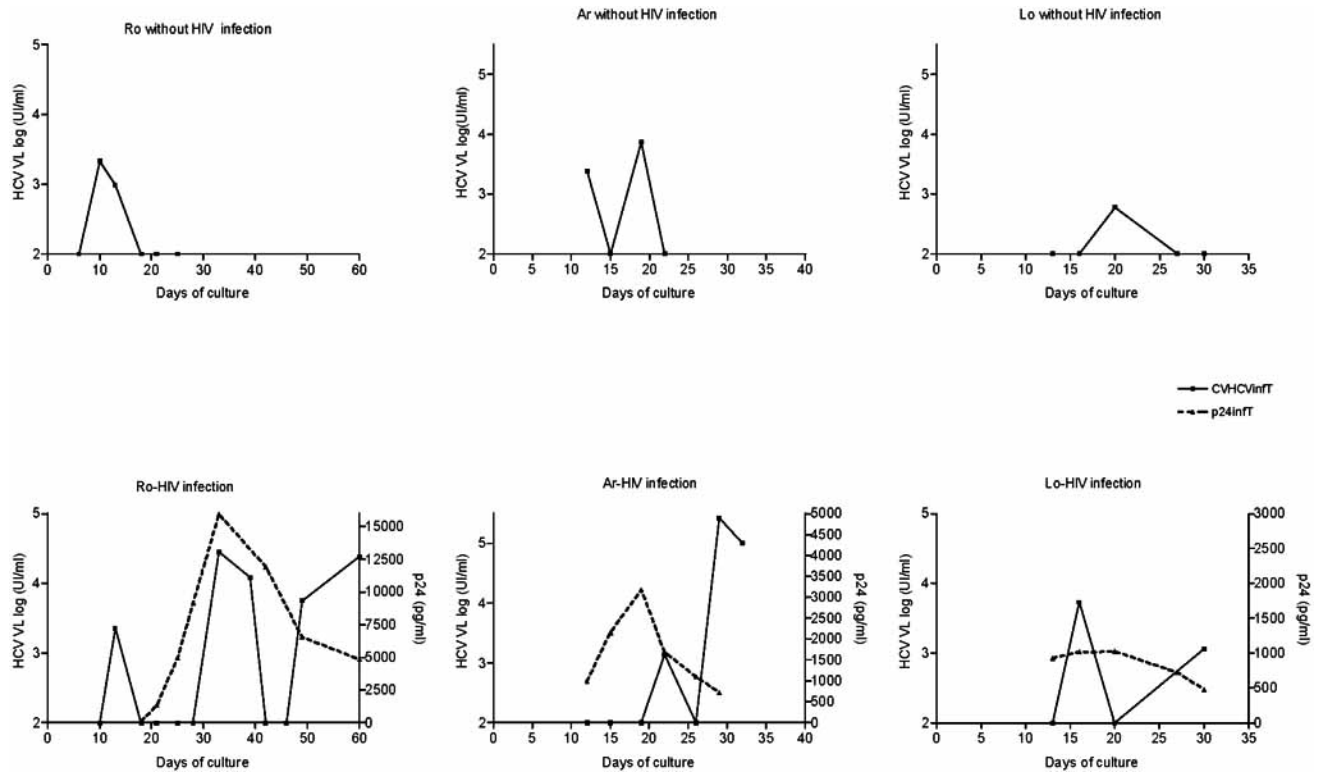


Fig. (3). Experimental infection of PBMC from HCV+ patients with HIV sources.

HCV RNA and HIV p24 release into the supernatant was assayed at different times after culture of HCV+ PBMC with (lower panels) or without addition of infectious HIV particles.

Other studies reporting possible interactions between both viruses were based, in general, on speculations on indirect clinical or immunological/virological parameter observations in coinfecting individuals [13, 24, 116, 142]. Due to the lack of a culture system that could support efficient HIV and HCV replication, a direct influence of HIV genes on HCV replication is more difficult to prove.

Some of the aspects of HIV/HCV interplay could be analyzed taking advantage of the possibility of performing long term culture of PBMCs from HIV and HCV/HIV patients in the absence of exogenously added stimuli [9, 119]. HIV coinfection influenced the frequency of HCV productive cultures derived from PBMCs and an enhancing effect of HIV on HCV persistence and replication *in vitro* was demonstrated. In the HIV group the probability of obtaining HCV replication in PBMC cultures was related to the CD4+ cell count and to the presence of HIV viremia. Low CD4 T cell counts affecting the magnitude and breadth of CD8 immunological control against HCV [66] may be the cause of the higher frequency of HCV+ cultures observed in the non responder group of patients. On the other hand, the presence of HIV viremia could be facilitating PBMC infection by HCV *in vivo* [74], resulting in an increased possibility of generating a positive HCV culture (more cells infected by HCV, more probability to escape from immunological control and give an HCV+ culture) [10].

In order to elucidate events of HIV direct or indirect impact we performed HIV infection assays on PBMC cul-

tures of HCV monoinfected patients. Production of HCV after HIV infection in culture was increased 1 or 2 logs compared to uninfected controls (Fig. 3). On the contrary, HIV infection didn't modify those cultures that were HCV non-producers. Nevertheless, lack of sensitivity of the assay could account for the absence of HCV RNA. These preliminary results suggest a direct HIV/HCV interaction, since HCV detection occurred after the peak of HIV maximum replication (Fig. 3). Although B cell activation, with the consequent HCV viral load increase, may be other cause of the observed changes after HIV infection in PBMC cultures, unspecific stimulus didn't account for the HCV viral burden increase. Taken together these results suggest that non stimulated PBMC culture provides a suitable system to obtain HCV replication in leukocytes *in vitro*. Under these conditions the influence of HIV on HCV infection could be addressed more directly.

Several questions need to be answered:

- 1) Does HIV directly enhance HCV replication or is this effect the consequence of HIV infection of other cell types that control HCV replication?
- 2) Do HIV and HCV infect the same cells *in vitro*?
- 3) What is the effect of HCV infection on HIV replication?
- 4) Are the experimental assays selective for a given HCV genotype, and if so, how does this correlate with the *in vivo* genotype balance?

While a lot of work needs to be performed, it will be encouraging to develop relatively simple systems to focus on some of these questions in order to try to understand the interplay of these viruses that frequently co-infect the same patients.

CONCLUSIONS

It is clear that HIV infection alters the pattern of progression of HCV disease, leading to increased mortality and morbidity in co-infected patients. Shared receptors for viral entrance (as DC-SIGN) and/or intracellular pathways, altered immune defense caused by HIV-induced immunosuppression as well as interference in presentation, antigen processing or effector cell function may be part of HIV/HCV interaction leading to increased HCV replication and persistence. However, it will be necessary to develop experimental systems that allow simultaneous cellular infection with the two viruses in order to determine if such an effect is a direct one. In this regard, the use of non stimulated PBMC culture could provide help to study the interaction of HCV with HIV, at least in this target cell system.

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REFERENCES

- [1] Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Gerber MA, Sampliner RE, Meeks EL, Beach MJ. (1992). The natural history of community-acquired hepatitis C in the United States. The Sentinel Counties Chronic non-A, non-B Hepatitis Study Team. *New England Journal of Medicine*. 327: 1899-1905.
- [2] Altfeld M, Rosenberg ES, Shankarappa R, Mukherjee JS, Hecht FM, Eldridge RL, Addo MM, Poon SH, Phillips MN, Robbins GK, Sax PE, Boswell S, Kahn JO, Brander C, Goulder PJ, Levy JA, Mullins JI, Walker BD. (2001). Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection. *Journal of Experimental Medicine*. 193: 169-180.
- [3] Anthony DD, Yonkers NL, Post AB, Asaad R, Heinzl FP, Lederman MM, Lehmann PV, Valdez H. (2004). Selective impairments in dendritic cell-associated function distinguish hepatitis C virus and HIV infection. *Journal of Immunology*. 172: 4907-16.
- [4] Appay V, Dunbar PR, Callan M, Klenerman P, Gillespie GM, Papagno L, Ogg GS, King A, Lechner F, Spina CA, Little S, Havlir DV, Richman DD, Gruener N, Pape G, Waters A, Easterbrook P, Salio M, Cerundolo V, McMichael AJ, Rowland-Jones SL. (2002). Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. *Nature Medicine*. 8: 379-385.
- [5] Appay V, Nixon DF, Donahoe SM, Gillespie G, Dong T, King A, Ogg GS, Spiegel HML, Conlon C, Spina CA, Havlir DV, Richman DD, Waters A, Easterbrook P, McMichael AJ, Rowland-Jones SL. (2000). HIV-specific CD8+ T cells produce antiviral cytokines but are impaired in their lytic function. *Journal of Experimental Medicine*. 192: 63-75.
- [6] Bain C, Fatmi A, Zoulim F, Zarski JP, Trepo C, Inchauspe G. (2001). Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection. *Gastroenterology* 120: 512-524.
- [7] Balasubramanian A, Ganju RK, Groopman JE. (2003). Hepatitis C virus and HIV envelope proteins collaboratively mediate interleukin-8 secretion through activation of p38 MAP kinase and SHP2 in hepatocytes. *Journal of Biological Chemistry*. 278: 35755-66
- [8] Banchereau J, Steinman RM. (1998). Dendritic cells and the control of immunity. *Nature*. 392: 245-252.
- [9] Bare P, Massud I, Belmonte L, Corti M, Villafane M, Perez Bianco R, de Tezanos-pinto M, de Bracco MM, Ruibal-Ares B. (2003). HCV recovery from peripheral blood mononuclear cell culture supernatants derived from HCV-HIV co-infected haemophilic patients with undetectable HCV viraemia. *Haemophilia*. 9: 598-604.
- [10] Bare P, Massud I, Parodi C, Belmonte L, Garcia G, Nebel MC, Corti M, Pinto MT, Bianco RP, Bracco MM, Campos R, Ares BR. (2005). Continuous release of hepatitis C virus (HCV) by peripheral blood mononuclear cells and B-lymphoblastoid cell-line cultures derived from HCV-infected patients. *Journal of General Virology*. 86: 1717-1727.
- [11] Bartenschlager R, Lohmann V. (2000). Replication of hepatitis C virus. *Journal of General Virology*. 81: 1631-1648.
- [12] Bashirova AA, Geijtenbeek TB, van Duijnhoven GC, van Vliet SJ, Eilering JB, Martin MP, Wu L, Martin TD, Viebig N, Knolle PA, KewalRamani VN, van Kooyk Y, Carrington M. (2001). A dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)-related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. *Journal of Experimental Medicine*. 193: 671-678.
- [13] Beld M, Penning M, Lukashov V, McMorrow M, Roos M, Pakker N, van den Hoek A, Goudsmit J. (1998). Evidence that both HIV and HIV-induced immunodeficiency enhance HCV replication among HCV seroconverters. *Virology*. 244: 504-512.
- [14] Benhamou Y, Bochet M, Di Martino V, Charlotte F, Azria F, Coutellier A, Vidaud M, Bricaire F, Opolon P, Katlama C, Poinard T. (1999). Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfecting patients. The Multivirc Group. *Hepatology*. 30: 1054-1058.
- [15] Betts MR, Nason MC, West SM, De Rosa C, Migueles SA, Abraham Lederman MM, Benito JM, Goepfert PA, Connors M, Roederer M, Koup RA. (2006). HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. *Blood*. 107: 4781-4789.
- [16] Bevan MJ, Fink PJ. (2001). The CD8 response on autopilot. *Nature Immunology*. 2: 381-382.
- [17] Blanchard E, Belouard S, Goueslain L, Wakita T, Dubuisson J, Wychowski C, Rouille Y. (2006). Hepatitis C virus entry depends on clathrin-mediated endocytosis. *Journal of Virology*. 80: 6964-72.
- [18] Blom B, Ho S, Antonenko S, Liu YJ. (2000). Generation of interferon alpha-producing dendritic cell (Pre-DC) 2 from human CD34 (+) hematopoietic stem cells. *Journal of Experimental Medicine*. 192: 1785-1795.
- [19] Burleigh L, Lozach P-Y, Schiffer C, Staropoli I, Pezo V, Porrot F, Canque B, Virelizier J-L, Aranzana-Seisdedos F, Amara A. (2006). Infection of dendritic cells (DCs), not DC-SIGN-mediated internalization of human immunodeficiency virus, is required for long-term transfer of virus to T cells. *Journal of Virology*. 80: 2949-57.
- [20] Cai Z, Zhang C, Chang KS, Jiang J, Ahn BC, Wakita T, Liang TJ, Luo G. (2005). Robust production of infectious hepatitis C virus (HCV) from stably HCV cDNA-transfected human hepatoma cells. *Journal of Virology*. 79:13963-73.
- [21] Chisari FV. (2005). Unscrubbing hepatitis C virus-host interactions. *Nature*. 436: 930-932.
- [22] Chung RT, Evans SR, Yang Y, Theodore D, Valdez H, Clark R, Shikuma C, Nevin T, Sherman KE; AIDS Clinical Trials Group 383 Study Team. (2002). Immune recovery is associated with persistent rise in hepatitis C virus RNA, infrequent liver test flares, and is not impaired by hepatitis C virus in co-infected subjects. *AIDS*. 16: 1915-1923.
- [23] Cribier B, Rey D, Schmitt C, Lang JM, Kirm A, Stoll-Keller F. (1995). High hepatitis C viraemia and impaired antibody response in patients coinfecting with HIV. *AIDS*. 9: 1131-1136.
- [24] Daar ES, Lynn H, Donfield S, Gomperts E, Hilgartner MW, Hoots WK, Chernoff D, Arkin S, Wong WY, Winkler CA. (2001). Relation between HIV-1 and hepatitis C viral load in patients with hemophilia. *Journal of Acquired Immune Deficiency Syndrome*. 26: 466-472.
- [25] Daar ES, Lynn H, Donfield S, Gomperts E, O'Brien SJ, Hilgartner MW, Hoots WK, Chernoff D, Arkin S, Wong WY, Winkler CA. (2001). Hepatitis C virus load is associated with human immunodeficiency virus type 1 disease progression in hemophiliacs. *Journal of Infectious Diseases*. 183: 589-595.
- [26] Darby SC, Ewart DW, Giangrande PL, Spooner RJ, Rizza CR, Dusheiko GM, Lee CA, Ludlam CA, Preston FE. (1997). Mortality

- from liver cancer and liver disease in haemophilic men and boys in UK given blood products contaminated with hepatitis C. UK Haemophilia Centre Directors' Organisation. *Lancet*. 350: 1425-1431.
- [27] Delladetsima J, Katsarou O, Touloumi G, Vgenopoulou S, Hatzakis A, Karafoulidou A. (2002). Significance of immune status, genotype and viral load in the severity of chronic hepatitis C in HIV infected haemophilia patients. *Haemophilia*. 8: 668-673.
- [28] Doherty PC. (1993). Immune exhaustion: driving specific CD8+ T cells to death. *Trends Microbiology*. 1: 207-209.
- [29] Donaghy H, Gazzard B, Gotch F, Patterson S. (2003). Dysfunction and infection of freshly isolated blood myeloid and plasmacytoid dendritic cells in patients infected with HIV-1. *Blood*. 101: 4505-4511.
- [30] Eisen-Vandervelde AL, Yao ZQ, Hahn YS. (2004). The molecular basis of HCV-mediated immune dysregulation. *Clinical Immunology*. 111: 16-21.
- [31] Eyster ME, Fried MW, Di Bisceglie AM, Goedert JJ. (1994). Increasing hepatitis C virus RNA levels in hemophiliacs: relationship to human immunodeficiency virus infection and liver disease. Multicenter Hemophilia Cohort Study. *Blood*. 84: 1020-1023.
- [32] Feldman S, Stein D, Amrute S, Denny T, Garcia Z, Kloser P, Sun Y, Megjugorac N, Fitzgerald-Bocarsly P. (2001). Decreased interferon-alpha production in HIV-infected patients correlates with numerical and functional deficiencies in circulating type 2 dendritic cell precursors. *Clinical Immunology*. 101: 201-210.
- [33] Ferbeyre G, Bourdeau V, Cedergren R. (1997). Does HIV tat protein also regulate genes of other viruses present in HIV infection? *Trends in Biochemical Sciences*. 22: 115-116.
- [34] Fialaire P, Payan C, Vitour D, Chennebault JM, Loison J, Pichard E, Lunel F. (1999). Sustained disappearance of hepatitis C viremia in patients receiving protease inhibitor treatment for human immunodeficiency virus infection. *Journal of Infectious Diseases*. 180: 574-575.
- [35] Flint M, Quinn ER, Levy S. (2001). Viral heterogeneity of the hepatitis C virus. *Clinics in Liver Disease*. 5: 873-893.
- [36] Francavilla V, Accapezzato D, De Salvo M, Rawson P, Cosimi O, Lipp M, Cerino A, Cividini A, Mondelli MU, Barnaba V. (2004). Subversion of CD8+ T cell differentiation in acute hepatitis C virus infection: exploring the immunological mechanisms. *European Journal of Immunology*. 34: 427-437.
- [37] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncalves FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. (2002). Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *New England Journal of Medicine*. 347: 975-982.
- [38] Gale M, Foy EM. (2005). Evasion of intracellular host defence by hepatitis C virus. *Nature*. 436: 939-945.
- [39] Garcia-Samaniego J, Rodriguez M, Berenguer J, Rodriguez-Rosado R, Carbo J, Asensi V, Soriano V. (2001). Hepatocellular carcinoma in HIV-infected patients with chronic hepatitis C. *The American Journal of Gastroenterology*. 96: 179-183.
- [40] Gardner JP, Durso RJ, Arrigale RR, Donovan GP, Maddon PJ, Dragic T, Olson WC. (2003). L-SIGN (CD 209L) is a liver-specific capture receptor for hepatitis C virus. *Proceedings of the National Academy of Sciences, United States of America*. 100: 4498-4503.
- [41] Geijtenbeek TB, Kwon DS, Torensma R, van Vliet SJ, van Duijnhoven GC, Middel J, Cornelissen IL, Nottet HS, KewalRamani VN, Littman DR, Figdor CG, van Kooyk Y. (2000). DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell*. 100: 587-597.
- [42] Gerlach JT, Diepolder HM, Jung MC, Gruener NH, Schraut WW, Zachoval R, Hoffmann R, Schirren CA, Santantonio T, Pape GR. (1999). Recurrence of hepatitis C virus after loss of virus-specific CD4(+) T-cell response in acute hepatitis. *Gastroenterology*. 117: 933-941.
- [43] Gerotto M, Sullivan DG, Polyak SJ, Chemello L, Cavalletto L, Pontisso P, Alberti A, Gretch DR. (1999). Effect of retreatment with interferon alone or interferon plus ribavirin on hepatitis C virus quasispecies diversification in nonresponder patients with chronic hepatitis C. *Journal of Virology*. 73: 7241-7247.
- [44] Goutagny N, Fatmi A, De Ledinghen V, Penin F, Couzigou P, Inchauspe G, Bain C. (2003). Evidence of viral replication in circulating dendritic cells during hepatitis C virus infection. *Journal of Infectious Diseases*. 187: 1951-1958.
- [45] Graham CS, Koziel MJ. (2000). Why should hepatitis C affect immune reconstitution in HIV-1 infected patients? *Lancet*. 356: 1865-1866.
- [46] Grakoui A, McCourt DW, Wychowski C, Feinstone SM, Rice CM. (1993). A second hepatitis C virus-encoded proteinase. *Proceedings of the National Academy of Sciences, United States of America*. 90: 10583-10587.
- [47] Greub G, Ledergerber B, Battegay M, Grob P, Perrin L, Furrer H, Burgisser P, Erb P, Boggian K, Piffaretti JC, Hirschel B, Janin P, Francioli P, Flepp M, Telenti A. (2000). Clinical progression, survival, and immune recovery during antiretroviral therapy in patients with HIV-1 and hepatitis C virus coinfection: the Swiss HIV Cohort Study. *Lancet*. 356: 1800-1805.
- [48] Groot F, van Capel TMM, Kapsenberg ML, Berkhout B, de Jong EC. (2006) Opposing roles of blood myeloid and plasmacytoid dendritic cells in HIV-1 infection of T cells: transmission facilitation versus replication inhibition. *Blood* prepublished online, may 16; DOI 10.1182/blood-2006-03-010918.
- [49] Grüner NH, Gerlach TJ, Jung MC, Diepolder HM, Schirren CA, Schraut WW, Hoffman R, Zachoval R, Santantonio T, Cucchiari M, Cerny A, Pape GR. (2000). Association of hepatitis C virus-specific CD8+ T cells with viral clearance in acute hepatitis C. *Journal of Infectious Diseases*. 181: 1528-1536.
- [50] Heath WR, Villadangos JA. (2005). No driving without a license. *Nature Immunology*. 6: 125-126.
- [51] Hijikata M, Mizushima H, Akagi T, Mori S, Kakiuchi N, Kato N, Tanaka T, Kimura K, Shimotohno K. (1993). Two distinct proteinase activities required for the processing of a putative nonstructural precursor protein of hepatitis C virus. *Journal of Virology*. 67: 4665-4675.
- [52] Hino K, Yamaguchi Y, Fujiwara D, Katoh Y, Korenaga M, Okazaki M, Okuda M, Okita K. (2000). Hepatitis C virus quasispecies and response to interferon therapy in patients with chronic hepatitis C: a prospective study. *Journal of Viral Hepatitis*. 7: 36-42.
- [53] Ikeda M, Sugiyama K, Mizutani T, Tanaka T, Tanaka K, Sekihara H, Shimotohno K, Kato N. (1998). Human hepatocyte clonal cell lines that support persistent replication of hepatitis C virus. *Virus Research*. 56: 157-167.
- [54] Ilan E, Arazi J, Nussbaum O, Zauberman A, Eren R, Lubin I, Neville L, Ben-Moshe O, Kischitzky A, Litchi A, Margalit I, Gopher J, Mounir S, Cai W, Daudi N, Eid A, Jurim O, Czerniak A, Galun E, Dagan S. (2002). The hepatitis C virus (HCV)-Trimer mouse: a model for evaluation of agents against HCV. *Journal of Infectious Diseases*. 185: 153-161.
- [55] Ishii N, Watashi K, Hishiki T, Goto K, Inoue D, Hijikata M, Wakita T, Kato N, Shimotohno K. (2006). Diverse effects of cyclosporine on hepatitis C virus strain replication. *Journal of Virology*. 80: 4510-20.
- [56] Ito T, Mukaigawa J, Zuo J, Hirabayashi Y, Mitamura K, Yasui K. (1996). Cultivation of hepatitis C virus in primary hepatocyte culture from patients with chronic hepatitis C results in release of high titre infectious virus. *Journal of General Virology*. 77: 1043-54.
- [57] Jinushi M, Takehara T, Tatsumi T, Kanto T, Groh V, Spies T, Suzuki T, Miyagi T, Hayashi N. (2003). Autocrine/paracrine IL-15 that is required for type I IFN-mediated dendritic cell expression of MHC class I-related chain A and B is impaired in hepatitis C virus infection. *Journal of Immunology*. 171: 5423-5429.
- [58] Kanda T, Basu A, Steele R, Wakita T, Ryerse JS, Ray R, Ray RB. (2006). Generation of infectious hepatitis C virus in immortalized human hepatocytes. *Journal of Virology*. 80: 4633-9.
- [59] Kanto T, Hayashi N, Takehara T, Tatsumi T, Kuzushita N, Ito A, Sasaki Y, Kasahara A, Hori M. (1999). Impaired allostimulatory capacity of peripheral blood dendritic cells recovered from hepatitis C-virus infected individuals. *Journal of Immunology*. 162: 5584-5591.
- [60] Kanto T, Hayashi N. (2006). Immunopathogenesis of hepatitis C virus infection: multifaceted strategies subverting innate and adaptive immunity. *Internal Medicine*. 45: 183-91.
- [61] Kanto T, Inoue M, Miyatake H, Sato A, Sakakibara M, Yakushijin T, Oki C, Itose I, Hiramatsu N, Takehara T, Kasahara A, Hayashi N. (2004). Reduced numbers and impaired ability of myeloid and plasmacytoid dendritic cells to polarize T helper cells in chronic hepatitis C virus infection. *Journal of Infectious Diseases*. 190: 1919-1926.
- [62] Kanto T, Inoue M, Miyazaki M, Itose I, Miyatake H, Sakakibara M, Yakushijin T, Kaimori A, Oki C, Hiramatsu N, Kasahara A,

- Hayashi N. (2006). Impaired function of dendritic cells circulating in patients infected with hepatitis C virus who have persistently normal alanine aminotransferase levels. *Intervirology*. 49: 58-63.
- [63] Kato N, Ikeda M, Mizutani T, Sugiyama K, Noguchi M, Hirohashi S, Shimotohno K. (1996). Replication of hepatitis C virus in cultured non-neoplastic human hepatocytes. *Japanese Journal of Cancer Research*. 87: 787-92.
- [64] Kato T, Date T, Miyamoto M, Furusaka A, Tokushige K, Mizokami M, Wakita T. (2003). Efficient replication of the genotype 2a hepatitis C virus subgenomic replicon. *Gastroenterology*. 125: 1808-1817.
- [65] Kawamura T, Furusaka A, Koziel MJ, Chung RT, Wang TC, Schmidt EV, Liang TJ. (1997). Transgenic expression of hepatitis C virus structural proteins in the mouse. *Hepatology*. 25:1014-1021.
- [66] Kim AY, Lauer GM, Ouchi K, Addo MM, Lucas M, Wiesch JS, Timm J, Boczanowski M, Duncan JE, Wurcel AG, Casson D, Chung RT, Draenert R, Klenerman P, Walker BD. (2005). The magnitude and breadth of hepatitis C virus-specific CD8+ T cells depend on absolute CD4+ T-cell count in individuals coinfecting with HIV-1. *Blood*. 105: 1170-1178.
- [67] Koike K, Moriya K, Ishibashi K, Matsuura Y, Suzuki T, Saito I, Iino S, Kurokawa K, Miyamura T. (1995). Expression of hepatitis C virus envelope proteins in transgenic mice. *Journal of General Virology*. 76: 3031-3038.
- [68] Koup R, Ho D, Poli G, Fauci AS. (1993). Isolation and quantitation of HIV in peripheral blood. In: *Current Protocols in Immunology*. (JE Coligan, AM Kruisbek, E Margulis, E Shevach and W Strober, Eds). Wiley New York. Suppl 5, 12.2.11
- [69] Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, Farthing C, Ho DD. (1994). Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *Journal of Virology*. 68: 4650-4655.
- [70] Krathwohl MD, Schacker TW, Anderson JL. (2006). Abnormal Presence of Semimature Dendritic Cells That Induce Regulatory T Cells in HIV-Infected Subjects. *Journal of Infectious Diseases*. 193: 494-504.
- [71] Kwon DS, Gregorio G, Bitton N, Hendrickson WA, Littman DR. (2002). DC-SIGN-mediated internalization of HIV is required for trans-enhancement of T cell infection. *Immunity*. 16: 135-144.
- [72] Lalvani A, Brookes R, Hambleton S, Britton WJ, Hill AV, McMichael AJ. (1997). Rapid effector function in CD8 memory T cells. *Journal of Experimental Medicine*. 186: 859-865.
- [73] Landau A, Batisse D, Piketty C, Duong Van Huyen JP, Bloch F, Belec L, Bruneval P, Weiss L, Jian R, Kazatchkine MD. (2001). Long-term efficacy of combination therapy with interferon-alpha 2b and ribavirin for severe chronic hepatitis C in HIV-infected patients. *AIDS*. 15: 2149-2155.
- [74] Laskus T, Radkowski M, Jablonska J, Kibler K, Wilkinson J, Adair D, Rakela J. (2004). Human immunodeficiency virus facilitates infection/replication of hepatitis C virus in native human macrophages. *Blood*. 103: 3854-9.
- [75] Laskus T, Radkowski M, Piasek A, Nowicki M, Horban A, Cianciara J, Rakela J. (2000). Hepatitis C virus in lymphoid cells of patients coinfecting with human immunodeficiency virus type 1: evidence of active replication in monocytes/macrophages and lymphocytes. *Journal of Infectious Diseases*. 181: 442-448.
- [76] Lauer GM, Walker BD. (2001). Hepatitis C virus infection. *New England Journal of Medicine*. 345: 41-52.
- [77] Lesens O, Deschenes M, Steben M, Belanger G, Tsoukas CM. (1999). Hepatitis C virus is related to progressive liver disease in human immunodeficiency virus-positive hemophiliacs and should be treated as an opportunistic infection. *Journal of Infectious Diseases*. 179: 1254-1258.
- [78] Lieberman J, Shankar P, Manjunath N, Andersson J. (2001). Dressed to kill? A review of why antiviral CD8 T lymphocytes fail to prevent progressive immunodeficiency in HIV-1 infection. *Blood*. 98: 1667-1677.
- [79] Lindenbach BD, Evans MJ, Syder AJ, Wolk B, Tellinghuisen TL, Liu CC, Maruyama T, Hynes RO, Burton DR, McKeating JA, Rice CM. (2005). Complete replication of hepatitis C virus in cell culture. *Science*. 309:623-6.
- [80] Lohmann V, Korner F, Koch J, Herian U, Theilmann L, Bartenschlager R. (1999). Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science*. 285: 110-113.
- [81] Lozach PY, Lortat-Jacob H, de Lacroix de Lavalette A, Staropoli I, Foug S, Amara A, Houles C, Fieschi F, Schwartz O, Virelizier JL, Arenzana-Seisdedos F, Altmeyer R. (2003). DC-SIGN and L-SIGN are high affinity binding receptors for hepatitis C virus glycoprotein E2. *Journal of Biological Chemistry*. 278: 20358-20366.
- [82] Lucas M, Vargas Cuero A, Lauer GM, Barnes E, Wilberg CB, Semmo N, Walker BD, Phillips R, Klenerman P. (2004). Pervasive influence of hepatitis C virus on the phenotype of antiviral CD8+ T cells. *Journal of Immunology*. 172: 1744-1753.
- [83] Makris M, Preston FE, Rosendaal FR, Underwood JC, Rice KM, Triger DR. (1996). The natural history of chronic hepatitis C in haemophiliacs. *British Journal of Haematology*. 94: 746-752.
- [84] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. (2001). Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet*. 358: 958-965.
- [85] Martínez-Sierra C, Arizcorreta A, Diaz F, Roldan R, Martín-Herrera L, Perez-Guzman E, Giron-Gonzalez JA. (2003). Progression of chronic hepatitis C to liver fibrosis and cirrhosis in patients coinfecting with hepatitis C virus and human immunodeficiency virus. *Clinical Infectious Diseases*. 36: 491-498.
- [86] Masso M. (2003). DC-SIGN points the way to a novel mechanism for HIV-1 transmission. *Medscape General Medicine*. 5: 2.
- [87] Matter M, Mumprecht S, Pinschewer DD, Pavelic V, Yagita H, Krautwald S, Borst J, Ochsenbein A. (2005). Virus-induced polyclonal B cell activation improves protective CTL memory *via* retained CD27 expression on memory CTL. *European Journal of Immunology*. 35: 3229-3239.
- [88] Matthews-Greer JM, Caldito GC, Adley SD, Willis R, Mire AC, Jamison RM, McRae KL, King JW, Chang WL. (2001). Comparison of hepatitis C viral loads in patients with or without human immunodeficiency virus. *Clinical and Diagnostic Laboratory Immunology*. 8: 690-694.
- [89] McDonald D, Wu L, Bohks SM, KewalRamani VN, Unutmaz D, Hope TJ. (2003). Recruitment of HIV and its receptors to dendritic cell-T cell junctions. *Science*. 300: 1295-1297.
- [90] Michelet C, Chaplain JM, Petsaris O, Arvieux C, Ruffault A, Lotteau V, Andre P. (1999). Differential effect of ritonavir and indinavir on immune response to hepatitis C virus in HIV-1 infected patients. *AIDS*. 13:1995-1996.
- [91] Migueles SA, Laborico AC, Shupert WL, Sabbaghian MS, Rabin R, Hallahan CW, van Baarle D, Kostense S, Miedema F, McLaughlin M, Ehler L, Metcalf J, Liu S, Connors M. (2002). HIV-specific CD8+T cell proliferation is coupled to perforin expression and is maintained in non progressors. *Nature Immunology*. 3: 1061-1068.
- [92] Miyamoto M, Kato T, Date T, Mizokami M, Wakita T. (2006). Comparison between subgenomic replicons of hepatitis C virus genotypes 2a (JFH-1) and 1b (Con1 NK5.1). *Intervirology*. 49: 37-43.
- [93] Mohty M, Vialle-Castellano A, Nunes JA, Isnardon D, Olive D, Gaugler B. (2003). IFN-alpha skews monocyte differentiation into Toll-like receptor 7-expressing dendritic cells with potent. *Journal of Immunology*. 171: 3385-3393.
- [94] Moreno L, Quereda C, Moreno A, Perez-Elias MJ, Antela A, Casado JL, Dronza F, Mateos ML, Barcina R, Moreno S. (2004). Pegylated interferon alpha2b plus ribavirin for the treatment of chronic hepatitis C in HIV-infected patients. *AIDS*. 18: 67-73.
- [95] Munshi N, Balasubramanian A, Koziel M, Ganju RK, Groopman JE. (2003). Hepatitis C and human immunodeficiency virus envelope proteins cooperatively induce hepatocytic apoptosis *via* an innocent bystander mechanism. *Journal of Infectious Diseases*. 188: 1192-204.
- [96] Musey L, Hughes J, Schacker T, Shea T, Corey L, McElrath MJ. (1997). Cytotoxic T cell responses, viral load and disease progression in early human immunodeficiency virus type 1 infection. *New England Journal of Medicine*. 337: 1267-1274.
- [97] Nobile C, Moris A, Porrot F, Sol-Foulon N, Schwartz O. (2003). Inhibition of human immunodeficiency virus type 1 Env-mediated fusion by DC-SIGN. *Journal of Virology*. 77: 5313-5323.
- [98] Ogg GS, Jin X, Bonhoeffer S, Dunbar PR, Nowak MA, Monard S, Segal JP, Cao Y, Rowland-Jones SL, Cerundolo V, Hurlley A, Markowitz M, Ho DD, Nixon DF, McMichael AJ. (1998). Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science*. 279: 2103-2106.

- [99] Pahwa R, Mc Closkey TW, Aroniadis O, Strbo N, Krishnan S, Pahwa S. (2006). CD8+ T cells in HIV disease exhibit cytokine receptor perturbation and poor T cell receptor activation but are responsive to γ -chain cytokine-driven proliferation. *The Journal of Infectious Diseases*. 193: 879-887.
- [100] Patterson S, Rae A, Hockey N, Gilmour J, Gotch F. (2001). Plasmacytoid dendritic cells are highly susceptible to human immunodeficiency virus type 1 infection and release infectious virus. *Journal of Virology*. 75: 6710-6713.
- [101] Perez-Olmeda M, Garcia-Samaniego J, Soriano V. (2000). Hepatitis C viraemia in HIV-HCV co-infected patients having immune restoration with highly active antiretroviral therapy. *AIDS*. 14: 212.
- [102] Perez-Olmeda M, Nunez M, Romero M, Gonzalez J, Castro A, Arribas JR, Pedreira J, Barreiro P, Garcia-Samaniego J, Martin-Carbonero L, Jimenez-Nacher I, Soriano V. (2003). Pegylated IFN-alpha2b plus ribavirin as therapy for chronic hepatitis C in HIV-infected patients. *AIDS*. 17: 1023-1028.
- [103] Pham TNQ, MacParland S, Malrooney PM, Cooksley H, Naoumov NV, Michalak TI. (2004). Hepatitis C Virus Persistence after Spontaneous or Treatment-Induced Resolution of Hepatitis C. *Journal of Virology*. 78: 5867-5874.
- [104] Piccioli D, Tavarini S, Nuti S, Colombatto P, Brunetto M, Bonino F, Ciccorossi P, Zorat F, Pozzato G, Comar C, Abrignani S, Wack A. (2005). Comparable functions of plasmacytoid and monocyte-derived dendritic cells in chronic hepatitis C patients and healthy donors. *Journal of Hepatology*. 42: 61-7.
- [105] Pohlmann S, Soilleux EJ, Baribaud F, Leslie GJ, Morris LS, Trowsdale J, Lee B, Coleman N, Doms RW. (2001). DC-SIGNR, a DC-SIGN homologue expressed in endothelial cells, binds to human and simian immunodeficiency viruses and activates infection in trans. *Proceedings of the National Academy of Sciences, United States of America*. 98: 2670-2675.
- [106] Pohlmann S, Zhang J, Baribaud F, Chen Z, Leslie GJ, Lin G, Granelli-Piperno A, Doms RW, Rice CM, McKeating JA. (2003). Hepatitis C virus glycoproteins interact with DC-SIGN and DC-SIGNR. *Journal of Virology*. 77: 4070-4080.
- [107] Probst HC, McCoy K, Okazaki T, Honjo T, van den Broek M. (2005). Resting dendritic cells induce peripheral CD8+ T cell tolerance through PD-1 and CTLA-4. *Nature Immunology*. 6: 280-286.
- [108] Puoti M, Bonacini M, Spinetti A, Putzolu V, Govindarajan S, Zaltron S, Favret M, Callea F, Gargiulo F, Donato F, Carosi G; HIV-HCV Coinfection Study Group. (2001). Liver fibrosis progression is related to CD4 cell depletion in patients coinfecting with hepatitis C virus and human immunodeficiency virus. *Journal of Infectious Diseases*. 183: 134-137.
- [109] Puoti M, Gargiulo F, Roldan EQ, Chiodera A, Palvarini L, Spinetti A, Zaltron S, Putzolu V, Zanini B, Favilli F, Turano A, Carosi G. (2000). Liver damage and kinetics of hepatitis C virus and human immunodeficiency virus replication during the early phases of combination antiretroviral treatment. *Journal of Infectious Diseases*. 181: 2033-2036.
- [110] Qurishi N, Kreuzberg C, Luchters G, Effenberger W, Kupfer B, Sauerbruch T, Rockstroh JK, Spengler U. (2003). Effect of antiretroviral therapy on liver-related mortality in patients with HIV and hepatitis C virus coinfection. *Lancet*. 362: 1708-1713.
- [111] Radkowski M, Gallegos-Orozco JF, Jablonska J, Colby TV, Walewska-Zielecka B, Kubicka J, Wilkinson J, Adair D, Rakela J, Laskus T. (2005). Persistence of hepatitis C virus in patients successfully treated for chronic hepatitis C. *Hepatology*. 41: 106-114.
- [112] Ragni MV, Bontempo FA. (1999). Increase in hepatitis C virus load in hemophiliacs during treatment with highly active antiretroviral therapy. *Journal of Infectious Diseases*. 180: 2027-2029.
- [113] Reis e Sousa C. (2004). Activation of dendritic cells: translating innate into adaptive immunity. *Current Opinion of Immunology*. 16: 21-25.
- [114] Rey D, Fritsch S, Schmitt C, Meyer P, Lang JM, Stoll-Keller F. (2001). Quantitation of hepatitis C virus RNA in saliva and serum of patients coinfecting with HCV and human immunodeficiency virus. *Journal of Medical Virology*. 63: 117-119.
- [115] Rinaldo CR Jr, Piazza P. (2004). Virus infection of dendritic cells: portal for host invasion and host defense. *Trends in Microbiology*. 12: 337-345.
- [116] Rockstroh JK, Spengler U, Sudhop T, Ewig S, Theisen A, Hammerstein U, Bierhoff E, Fischer HP, Oldenburg J, Brackmann HH, Sauerbruch T. (1996). Immunosuppression may lead to progression of hepatitis C virus-associated liver disease in hemophiliacs coinfecting with HIV. *The American Journal of Gastroenterology*. 91: 2563-2568.
- [117] Rodriguez-Rosado R, Garcia-Samaniego J, Soriano V. (1998). Hepatotoxicity after introduction of highly active antiretroviral therapy. *AIDS*. 12: 1256.
- [118] Rowland-Jones S, Sutton J, Ariyoshi K, Dong T, Gotch F, McAdam S, Whitby D, Sabally S, Gallimore A, Corrah T, Takaguchi M, Schultz T, McMichael A, Whittle H. (1995). HIV-specific cytotoxic T cells in HIV-exposed but uninfected Gambian women. *Nature Medicine*. 1: 59-64.
- [119] Ruibal-Ares B, Belmonte L, Bare P, Bayo-Hanza C, Mendez G, Bianco RP, de Tezanos Pinto M, de Bracco MM. (2001). Monocyte differentiation and HIV replication after prolonged culture of peripheral blood mononuclear cells from HIV-infected individuals. *Cellular Immunology*. 210: 11-20.
- [120] Rullier A, Trimoulet P, Neau D, Bernard PH, Foucher J, Lacoste D, Wincock M, Urbaniak R, Ballardini G, Balabaud C, Bioulac-Sage P, Le Bail B. (2004). Fibrosis is worse in HIV-HCV patients with low-level immunodepression referred for HCV treatment than in HCV-matched patients. *Human Pathology*. 35: 1088-1094.
- [121] Rumin S, Berthillon P, Tanaka E, Kiyosawa K, Traubad MA, Bizollon T, Gouillat C, Gripon P, Gugen-Guillouzo C, Inchauspe G, Trepo C. (1999). Dynamic analysis of hepatitis C virus replication and quasispecies selection in long-term cultures of adult human hepatocytes infected *in vitro*. *Journal of General Virology*. 80: 3007-18.
- [122] Rutschmann OT, Negro F, Hirschel B, Hadengue A, Anwar D, Perrin LH. (1998). Impact of treatment with human immunodeficiency virus (HIV) protease inhibitors on hepatitis C viremia in patients coinfecting with HIV. *Journal of Infectious Diseases*. 177: 783-785.
- [123] Sabin CA, Telfer P, Phillips AN, Bhagani S, Lee CA. (1997). The association between hepatitis C virus genotype and human immunodeficiency virus disease progression in a cohort of hemophilic men. *Journal of Infectious Diseases*. 175: 164-168.
- [124] Sakaguchi S. (2004). Naturally arising CD4 regulatory T cells for immunologic self tolerance and negative control of immune responses. *Annual Review of Immunology*. 22: 531-562.
- [125] Sanchez-Quijano A, Andreu J, Gavilan F, Luque F, Abad MA, Soto B, Munoz J, Aznar JM, Leal M, Lissen E. (1995). Influence of human immunodeficiency virus type 1 infection on the natural course of chronic parenterally acquired hepatitis. *European Journal of Clinical Microbiology and Infectious Diseases*. 14: 949-953.
- [126] Schmitt N, Nugeyre MT, Scott-Algara D, Cumont MC, Barré-Sinoussi F, Pancino G, Israël N. (2006). Differential susceptibility of human thymic dendritic cell subsets to X4 and R5 HIV-1 infection. *AIDS*. 20: 533-542.
- [127] Seder RA, Ahmed R. (2003). Similarities and differences in CD4+ and CD8+ effector and memory T cell generation. *Nature Immunology*. 4: 835-842.
- [128] Seeff LB. (1999). Natural history of hepatitis C. *The American Journal of Medicine*. 107: 10S-15S.
- [129] Sherman KE, O'Brien J, Gutierrez AG, Harrison S, Urdea M, Neuwald P, Wilber J. (1993). Quantitative evaluation of hepatitis C virus RNA in patients with concurrent human immunodeficiency virus infections. *Journal of Clinical Microbiology*. 31: 2679-2682.
- [130] Shi Z, Wakil AE, Rockey DC. (1997). Strain-specific differences in mouse hepatic wound healing are mediated by divergent T helper cytokine responses. *Proceedings of the National Academy of Sciences, United States of America*. 94: 10663-10668.
- [131] Sieg SF, Harding CV, Lederman MM. (2001). HIV-1 infection impairs cell cycle progression of CD4(+) T cells without affecting early activation responses. *Journal of Clinical Investigation*. 108: 757-764.
- [132] Siegal FP, Lopez C, Fitzgerald PA, Shah K, Baron P, Leiderman IZ, Imperato D, Landesman S. (1986). Opportunistic infections in acquired immune deficiency syndrome result from synergistic defects of both the natural and adaptive components of cellular immunity. *Journal of Clinical Investigation*. 78: 115-123.
- [133] Simmonds P. (1999). Viral heterogeneity of the hepatitis C virus. *Journal of Hepatology*. Suppl 1: 54-60.
- [134] Soriano V, Nunez M, Camino N, Maida I, Barreiro P, Romero M, Martin-Carbonero L, Garcia-Samaniego J, Gonzalez-Lahoz J. (2004). Hepatitis C virus-RNA clearance in HIV-coinfected patients with chronic hepatitis C treated with pegylated interferon plus ribavirin. *Antiviral Therapy*. 9: 505-509.

- [135] Soto B, Sanchez-Quijano A, Rodrigo L, del Olmo JA, Garcia-Bengoechea M, Hernandez-Quero J, Rey C, Abad MA, Rodriguez M, Sales Gilabert M, Gonzalez F, Miron P, Caruz A, Relimpio F, Torronteras R, Leal M, Lissen E. (1997). Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. *Journal of Hepatology*. 26: 1-5.
- [136] Soumelis V, Scott I, Gheyas F, Bouhour D, Cozon G, Cotte L, Huang L, Levy JA, Liu YJ. (2001). Depletion of circulating natural type 1 interferon-producing cells in HIV-infected AIDS patients. *Blood*. 98: 906-912.
- [137] Sporri R, Reis e Sousa C. (2005). Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4+ T cell populations lacking helper function. *Nature Immunology*. 6: 163-170.
- [138] Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. (1998). How cells respond to interferons. *Annual Review of Biochemistry*. 67: 227-264.
- [139] Sulkowski MS, Mast EE, Seeff LB, Thomas DL. (2000). Hepatitis C virus infection as an opportunistic disease in persons infected with human immunodeficiency virus. *Clinical Infectious Diseases*. 30: S77-84.
- [140] Sulkowski MS, Moore RD, Mehta SH, Chaisson RE, Thomas DL. (2002). Hepatitis C and progression of HIV disease. *Journal of the American Medical Association*. 288: 199-206.
- [141] Thimme R, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. (2001). Determinants of viral clearance and persistence during acute hepatitis C virus infection. *Journal of Experimental Medicine*. 194: 1395-1406.
- [142] Thomas DL, Astemborski J, Rai RM, Anania FA, Schaeffer M, Galai N, Nolt K, Nelson KE, Strathdee SA, Johnson L, Laeyendecker O, Boitnott J, Wilson LE, Vlahov D. (2000). The natural history of hepatitis C virus infection: host, viral, and environmental factors. *Journal of the American Medical Association*. 284: 450-456.
- [143] Thomas DL, Astemborski J, Vlahov D, Strathdee SA, Ray SC, Nelson KE, Galai N, Nolt KR, Laeyendecker O, Todd JA. (2000). Determinants of the quantity of hepatitis C virus RNA. *Journal of Infectious Diseases*. 181: 844-851.
- [144] Thomas DL, Rich JD, Schuman P, Smith DK, Astemborski JA, Nolt KR, Klein RS. (2001). Multicenter evaluation of hepatitis C RNA levels among female injection drug users. *Journal of Infectious Diseases*. 183: 973-976.
- [145] Thomas DL, Shih JW, Alter HJ, Vlahov D, Cohn S, Hoover DR, Cheung L, Nelson KE. (1996). Effect of human immunodeficiency virus on hepatitis C virus infection among injecting drug users. *Journal of Infectious Diseases*. 174: 690-695.
- [146] Tural C, Fuster D, Tor J, Ojanguren I, Sirera G, Ballesteros A, Lasanta JA, Planas R, Rey-Joly C, Clotet B. (2003). Time on antiretroviral therapy is a protective factor for liver fibrosis in HIV and hepatitis C virus (HCV) co-infected patients. *Journal of Viral Hepatitis*. 10: 118-125.
- [147] Turville S, Wilkinson J, Cameron P, Dable J, Cunningham AL. (2003). The role of dendritic cell C-type lectin receptors in HIV pathogenesis. *Journal of Leukocyte Biology*. 74: 710-718.
- [148] Turville SG, Cameron PU, Handley A, Lin G, Pohlmann S, Doms RW, Cunningham AL. (2002). Diversity of receptors binding HIV on dendritic cell subsets. *Nature Immunology*. 3: 975-983.
- [149] Vento S, Garofano T, Renzini C, Casali F, Ferraro T, Concia E. (1998). Enhancement of hepatitis C virus replication and liver damage in HIV-coinfected patients on antiretroviral combination therapy. *AIDS*. 12: 116-117.
- [150] Villano SA, Vlahov D, Nelson KE, Cohn S, Thomas DL. (1999). Persistence of viremia and the importance of long-term follow-up after acute hepatitis C infection. *Hepatology*. 29: 908-914.
- [151] Vogel J, Hinrichs SH, Reynolds RK, Luciw PA, Jay G. (1988). The HIV tat gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. *Nature*. 335: 606-611.
- [152] Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Krausslich HG, Mizokami M, Bartenschlager R, Liang TJ. (2005). Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nature Medicine*. 11:791-6.
- [153] Walker CM, Moody DJ, Stites DP, Levy JA. (1986). CD8+ lymphocytes can control HIV infection *in vitro* by suppressing virus replication. *Science*. 234: 1563-1566.
- [154] Wedemeyer H, He X-S, Nascimbeni M, Davis AR, Greenberg HB, Hoofnagle JH, Liang TK, Alter H, Rehermann B. (2002). Impaired effector function of hepatitis C virus-specific CD8+ T cells in chronic hepatitis C virus infection. *Journal of Immunology*. 169: 3447-3458.
- [155] Wieland SF, Chisari FV. (2005). Stealth and cunning: hepatitis B and hepatitis C viruses. *Journal of Virology*. 79: 9369-9380.
- [156] Willflingseder D, Banki Z, Dierich MP, Stoiber H. (2005). Mechanisms promoting dendritic cell-mediated transmission of HIV. *Molecular Immunology*. 42: 229-237.
- [157] Wunschmann S, Medh JD, Klinzmann D, Schmidt WN, Stapleton JT. (2000). Characterization of hepatitis C virus (HCV) and HCV E2 interactions with CD81 and the low-density lipoprotein receptor. *Journal of Virology*. 74:10055-10062.
- [158] Yewdell JW, Bennink JR. (1999). Mechanisms of viral interference with MHC class I presentation. *Annual Review of Cell and Developmental Biology*. 15: 579-606.
- [159] Yi M, Villanueva RA, Thomas DL, Wakita T, Lemon SM. (2006). Production of infectious genotype 1a hepatitis C virus (Hutchinson strain) in cultured human hepatoma cells. *Proceedings of the National Academy of Sciences, United States of America*. 14;103: 2310-5.
- [160] Yokozaki S, Takamatsu J, Nakano I, Katano Y, Toyoda H, Hayashi K, Hayakawa T, Fukuda Y. (2000). Immunologic dynamics in hemophiliac patients infected with hepatitis C virus and human immunodeficiency virus: influence of antiretroviral therapy. *Blood*. 96: 4293-4299.
- [161] Yonezawa A, Morita R, Takaori-Kondo A, Kadowaki N, Kitawaki T, Hori T, Uchiyama T. (2003). Natural alpha interferon-producing cells respond to human immunodeficiency virus type 1 with alpha interferon production and maturation into dendritic cells. *Journal of Virology*. 77: 3777-3784.
- [162] Zajac AJ, Blattman JN, Murali-Krishna K, Sourdive DJ, Suresh M, Altman JD, Ahmed R. (1998). Viral immune evasion due to persistence of activated T cells without effector function. *The Journal of Experimental Medicine*. 188: 2205-2213.
- [163] Zhang J, Randall G, Higginbottom A, Monk P, Rice CM, McKeating JA. (2004). CD81 is required for hepatitis C virus glycoprotein-mediated viral infection. *Journal of Virology*. 78: 1448-1455.
- [164] Zhong J, Gastaminza P, Cheng G, Kapadia S, Kato T, Burton DR, Wieland SF, Uprichard SL, Wakita T, Chisari FV. (2005). Robust hepatitis C virus infection *in vitro*. *Proceedings of the National Academy of Sciences, United States of America*. 102: 9294-9.