A look behind closed doors: interaction of persistent viruses with dendritic cells

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Abstract | Persistent infections with HIV, hepatitis B virus and hepatitis C virus are major causes of morbidity and mortality worldwide. As sentinels of the immune system, dendritic cells (DCs) are crucial for the generation of protective antiviral immunity. Recent advances in our understanding of the role of DCs during infection with these viruses provide insights into the mechanisms used by these viruses to exploit DC function and evade innate and adaptive immunity. In this Review we highlight the current knowledge about the interaction between DCs and these viruses and the underlying mechanisms that might influence the outcome of viral infections.

The immune response to viral infection is a complex interplay between the virus and the innate and adaptive immune responses and is aimed at eradicating the pathogen with minimal damage to the host. Dendritic cells (DCs) are a specialized family of antigen-presenting cells (APCs) that effectively link the innate recognition of viruses to the generation of the appropriate type of adaptive immune response[1]. DCs are continuously produced from haematopoietic stem cells in the bone marrow and are positioned at the different portals of the human body, such as the skin, mucosal surfaces and the blood, so that they encounter invading pathogens early in the course of an infection[2].

DCs are a heterogeneous family. This heterogeneity arises at several levels, including phenotype, function and anatomical location[2] (TABLE 1). Langerhans cells form a long-lived population of stellate DCs in the epidermis; interstitial DCs comprise the DCs found in all peripheral tissues, excluding Langerhans cells. The haematopoietic stem cells also give rise to two other DC subsets in the blood: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). DCs are equipped with a set of varied pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), through which they sense and process viral information and become activated (TABLE 1). Following activation, DCs migrate to the regional lymph nodes, where they become mature interdigitating DCs in the T cell-dependent areas. As a result of viral-antigen uptake and presentation on the surface, in complex with major histocompatibility complex (MHC) class I and II molecules, DCs trigger an immune response in any T cell that possesses a cognate receptor specific for the viral-peptide–MHC molecule complexes being presented on the DC surface[1].

The different DC subsets seem to have evolved over time to acquire both distinct and overlapping functions in order to better defend the host. Both mDCs and pDCs function in innate and adaptive immunity and provide a critical link between the two arms of immunity that respond to viral infection[3]. Following activation, mDCs produce interleukin-12 (IL-12) and IL-15, which in turn stimulate interferon-γ (IFNγ) secretion by natural killer (NK) cells and promote the differentiation of CD4+ T helper (T_H) cells into T_H1 cells and of CD8+ T cells into cytotoxic T lymphocytes; these cells contribute to viral clearance by killing infected cells either directly, through the release of cytolytic mediators such as granzyme, or indirectly, by secreting T_H1-type cytokines that inhibit viral replication (FIG. 1). In contrast to mDCs, which may have evolved mainly to prime and activate antiviral T cells, pDCs are the key effector cells in the early antiviral innate immune response, as they produce large amounts of type I interferon on viral infection. Type I interferons (such as IFNα and IFNβ) released by pDCs not only have potent antiviral activity but also support subsequent steps of antiviral immunity, including the activation of NK cell-mediated cytotoxicity and CD4+ T cell and CD8+ T cell differentiation and survival[4]. In addition, pDCs also have an overlapping role as APCs[5].

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Table 1 | **Subsets of human dendritic cells**

		Myeloid subtypes			
	Plasmacytoid DCs	**Myeloid DCs**	**Langerhans cells**	**Interstitial DCs**	**Monocyte-derived DCs**
Morphology					
Localization	Blood	Blood	Epidermis	Dermis and other tissues	*In vitro*
Phenotype	$CD11c^{-}CD1a^{+}$ $CD1c^{-}CD123^{high}$ $CD304^{+}$	$CD11c^{+}CD1a^{+}$ $CD1c^{+}CD123^{low}$ $CD304^{-}$	$CD11c^{+}CD1a^{+}$ $CD207^{+}$	$CD11c^{+}CD1a^{-}$ $CD68^{+}$ and expresses coagulation factor XIII A chain	$CD11c^{+}CD1a^{+}$ $CD1c^{+}CD123^{low}$
TLR expression	TLR1, TLR6, TLR7, TLR9 and TLR10	TLR1,TLR2, TLR3, TLR4, TLR5, TLR6, TLR8 and TLR10	TLR1, TLR2, TLR3, TLR6, TLR7 and TLR8	TLR1,TLR2, TLR3, TLR4, TLR5, TLR6, TLR7 and TLR8,	TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR8 and TLR10
C-type lectin expression	BDCA2 and DCIR	DCIR, DC-SIGN and MR	CD207	DC-SIGN and MR	DCIR, DC-SIGN and MR
$CD4^{+}$ T cell priming	Yes	Yes	Yes	Yes	Yes
$CD8^{+}$ T cell priming	Yes	Yes	Yes	Yes	Yes
B cell activation	Yes	Yes	Yes (weak)	Yes	Yes
IFNα production	Yes (high)	Yes	Yes	Yes	Yes

BDCA2, blood DC antigen 2 (also known as CLEC4C); DC, dendritic cell; DC-SIGN, DC-specific ICAM3-grabbing non-integrin (also known as CD209); DCIR, DC immunoreceptor (also known as CLEC4A); IFNα, interferon-α; MR, mannose receptor; TLR, Toll-like receptor.

The importance of DCs in the clearance of viral infection has been shown for several viruses, such as the common respiratory viral pathogens human respiratory syncytial virus (HRSV) and influenza virus[6,7]. DCs also play an important part in the control of blood-borne viruses, the most common and deadly being hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency viruses (HIV-1 and HIV-2). Patients who spontaneously clear HBV and HCV infections or control HIV infection exhibit a strong multi-epitope-specific $CD4^{+}$ T cell and $CD8^{+}$ T cell response that probably reflects efficient priming and activation of antiviral T cells by DCs[8–11]. However, viral clearance after HBV, HCV or HIV infection is not always possible, and together these viruses have created a global health problem of substantial proportions. Not only do they establish asymptomatic persistent infections with potential oncogenic sequelae, but they also cause substantial morbidity and mortality (TABLE 2). HIV infection causes AIDS, which is characterized by profound immunosuppression and a diverse range of associated opportunistic infections[12]. Worldwide, HBV and HCV have infected more than 370 and 130 million people, respectively[13], and are the two major causes of chronic liver disease and its associated complications (including liver cirrhosis, liver failure and hepatocellular carcinoma)[14]. A common denominator in all these persistent infections is the weak and narrowly focused antiviral T cell response[8–11]. Owing to their central role in the initiation of the antiviral immune response, DCs are ideal targets for viruses to exercise their immune evasion strategies; in fact, viruses that cause persistent infection seem to have perfected the art of evading the pathogen recognition and elimination properties of DCs (BOX 1). Gaining a clearer understanding of these mechanisms in virus-infected DCs might enable us to better comprehend virus–host interactions and, in turn, might provide new perspectives for the therapy of persistent infections as well as for the design of vaccines.

This Review highlights the latest advances in our understanding of the interplay between DCs and these viruses that cause persistent viral infections. We focus on the interaction of HBV, HCV and HIV with different subtypes of DCs, outlining diverse outcomes of the virus–DC interaction and its relevance to viral pathogenesis as well as the mechanisms that these viruses have developed to interfere with the normal immune response of the host.

Do persistent viruses infect dendritic cells?

The presence of DCs in the skin, in the blood and, particularly, at the mucosal surfaces and their ability to take up antigen at these sites predisposes DCs to be primary target cells for viruses. It is therefore possible that viruses establish persistence by directly infecting DCs. It is not unreasonable to assume that replication of the viral genome, along with the expression of viral antigens, would interfere with signalling pathways in DCs

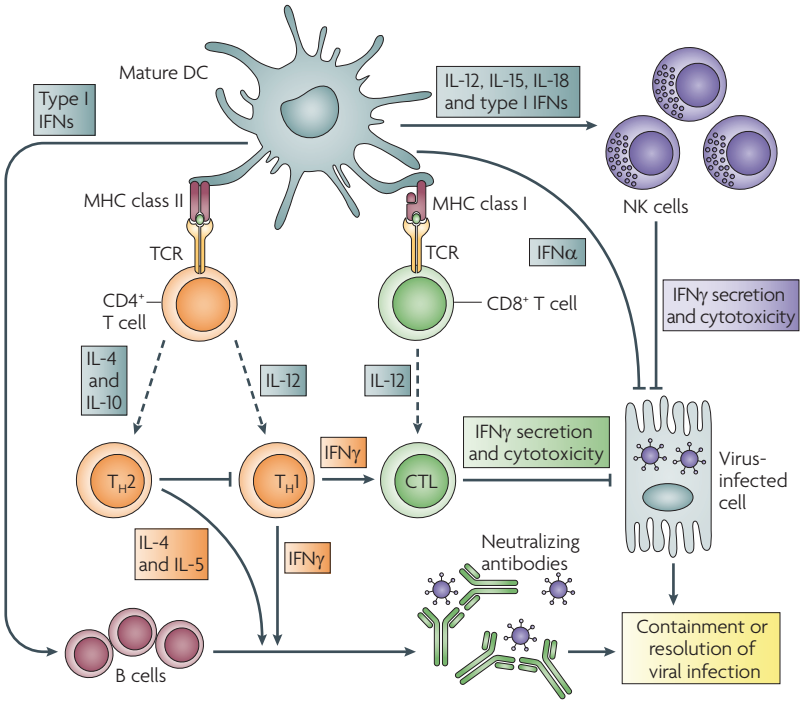

Figure 1 | **Function of dendritic cells in the immune response to viruses.** Following the uptake of viral antigen, myeloid dendritic cells (mDCs) and plasmacytoid DCs (pDCs) migrate to lymphoid tissue to prime naive CD4+ T cells and CD8+ T cells. In addition, activated DCs produce a range of cytokines, such as interferon-α (IFNα), interleukin-12 (IL-12) and IL-15, which in turn activate natural killer (NK) cells and influence T cell survival and differentiation. Depending on the cytokine signal, CD4+ T cells differentiate into T helper 1 (T_H1) or T_H2 cells (dashed arrows). T_H1 cell-mediated IFNγ secretion stimulates the activation of cytotoxic T lymphocytes (CTLs) and the production of immunoglobulin G2a antibodies by B cells. T_H2 cell-mediated cytokine production simulates immunoglobulin G1 antibody production by B cells but also inhibits activation of T_H1 cells. Virus-specific antibodies can be neutralizing, preventing viral reinfection. NK cells and CTLs inhibit viral replication through the secretion of IFNγ or through the lysis of virus-infected cells by releasing cytolytic mediators (namely, perforin and granzymes). In addition, pDCs are characterized by their ability to produce large amounts of type I IFNs in response to many viruses and thereby produce a first strong wave of IFNα, which not only inhibits viral replication but is also a potent enhancer for NK cell-mediated cytoxicity. Boxes are coloured the same as the cell that produces the cytokine or the effect. MHC, major histocompatibility complex. TCR, T cell receptor.

or directly impair DC function, rendering infected DCs less able to stimulate T cell responses. For example, ICP47 of herpes simplex virus 1 (HSV1) and US6 of human herpesvirus 5 (HHV5) are known to inhibit loading of antigenic peptides onto MHC class I molecules, thereby interfering with the ability of infected DCs to prime naive T cells efficiently[15].

HIV. Langerhans cells, the APCs of the epidermis, were the first DCs reported to be susceptible to HIV-1 infection. Since then, mDCs and pDCs isolated from the blood of patients infected with HIV-1 have been shown to be infected by the virus (reviewed in REF 16). However, HIV-1 replication in DCs is usually less productive, and the frequency of HIV-1-infected DCs *in vivo* is often 10–100 times lower than that of HIV-1-infected CD4+ T cells[17]. *In vitro* studies indicate that, on average, only 1–3% of mDCs and pDCs from healthy blood donors can be productively infected by primary HIV-1 and by laboratory-adapted HIV-1, as detected by intracellular staining of capsid protein p24 from HIV-1[18]. Immature DCs have been reported to be more susceptible to productive infection than mature DCs[19], which can be partly explained by the enhanced capacity of immature DCs to acquire viral antigen. During maturation of DCs, their ability to capture antigens through macropinocytosis and receptor-mediated endocytosis rapidly declines and, instead, the DCs assemble complexes of antigen with either MHC class I or MHC class II molecules[1]. Furthermore, HIV replication in pDCs was observed to increase substantially following CD40 ligation[20] (a signal that is delivered physiologically by CD4+ T cells). Thus, HIV replication in pDCs may be triggered through the interaction with activated CD4+ T cells in the extrafollicular T cell zones of the lymphoid tissue, suggesting that pDCs serve as viral reservoirs for CD4+ T cells.

HCV. Genomic RNA from HCV has been detected in pDCs and mDCs that were isolated from the blood of patients infected with the virus[21,22], and so it was initially thought that DCs were susceptible to HCV infection. However, using a strand-specific semi-quantitative reverse transcription PCR (RT-PCR), the replicative intermediate was observed in DCs isolated from only 3 out of 24 patients infected with HCV[21], indicating that HCV replication occurs at a lower frequency in DCs than in hepatocytes, which are the main site of HCV replication. To study HCV infection of DCs *in vitro*, monocyte-derived DCs from healthy individuals were incubated with serum from HCV-infected patients. The replicative intermediate was subsequently detected in these DCs, indicating that they may support at least the first steps of the viral life cycle[23]. However, following incubation of monocyte-derived DCs and subsets of blood DCs with infectious recombinant HCV (BOX 2), neither viral replication nor viral protein synthesis could be detected[24–28], suggesting that HCV may infect DCs but does not result in a productive infection.

HBV. Although the detection of HBV DNA in subsets of isolated blood DCs from patients infected with HBV has been proposed to indicate HBV infection of DCs[29], additional studies have not revealed the presence of RNA replicative intermediates in either blood DC subsets from patients infected with HBV or in DCs infected *in vitro* with wild-type or recombinant HBV[30,31]. Thus, it is likely that DCs do not support the replication and production of HBV viral particles and that the detection of HBV DNA merely reflects the attachment of the virus to the cell surface or the natural antigen uptake function of DCs.

In summary, DCs can support the production of HIV particles, although at a much lower level than is supported by CD4+ T cells (which are the primary targets for HIV), but cannot support the production of HCV and HBV particles, even though HCV may be able to initiate replication. There are three possible explanations for this.

Table 2 | **Clinical and virological features of hepatitis B virus, hepatitis C virus and HIV**

Characteristic	HBV	HCV	HIV
Structure	42 nm, enveloped nucleocapsid with a partially double-stranded DNA genome	50 nm, enveloped nucleocapsid with a positive single-stranded RNA genome	120 nm, enveloped nucleocapsid with a positive single-stranded RNA genome
Family	*Hepadnaviridae*	*Flaviviridae*	*Retroviridae*
Entry factors	Unknown	Glycosaminoglycans, CD81, SRB1, claudin 1 and occludin	CD4, CCR5, CXCR4 and DC-SIGN
Replication strategy	Reverse transcription of HBV RNA into a covalently closed circular DNA that serves as a template for HBV transcripts, which can then be reverse transcribed to make genomic DNA	Synthesis of a negative-strand RNA (using the viral genome as a template), which is then used as a template for synthesis of complementary positive-strand genomic RNA	Conversion of the single-stranded genomic RNA to double-stranded DNA by the viral reverse transcriptase followed by integration of the DNA into the host genome and, on activation, transcription of a full-length viral genomic RNA
Mutation rate	Low (1 in 100,000 bases per year)	High (1 in 1,000 bases per year)	High (1 in 10,000 bases per replication cycle)
Genotypes	8 genotypes (A–H) with 22 subtypes	6 main genotypes (1–6) with several subtypes (more than 50 in total)	HIV-1 (which comprises one major group, M, that is divided into nine subtypes (A, B, C, D, F, G, H, J and K) and two minor groups, O and N) and HIV-2 (which comprises two groups, A and B)
Transmission	Through intravenous drug use, blood transfusions, perinatal transfer and sexual contact	Through intravenous drug use, blood transfusions and perinatal transfer	Through intravenous drug use, blood transfusions, perinatal transfer and sexual contact
Infection site	Liver	Liver	$CD4^+$ T cells
Impact on public health			
Chronically infected individuals wordwide	370 million	130 million	35 million
New infections per year	4 million	3 to 4 million	3 million
Related deaths per year	500,000 to 1.2 million	350,000	2 million
Rate of co-infection with HCV	10% to 30%	NA	10%
Outcome of infection			
Spontaneous recovery	90% of adults and 10% of children	20%	0%
Disease	Liver cirrhosis (in 2% to 5% of chronically infected patients) and hepatocellular carcinoma (in 5% of patients with liver cirrhosis)	Liver cirrhosis (in 20% to 30% of chronically infected patients) and hepatocellular carcinoma (in 5% of patients with liver cirrhosis)	AIDS and susceptibility to life-threatening opportunistic infections
Available therapy	Nucleoside and nucleotide analogues and interferon-α, (which result in efficient control of viral infection) and liver transplantation with prevention of graft reinfection using antiviral treatment and HBsAg-specific antibodies	Ribavirin in combination with pegylated interferon-α1 (which leads to HCV clearance in 50% to 80% of individuals, depending on the HCV genotype) and liver transplantation (which can be beset by systematic reinfection of the graft)	Highly active antiretroviral therapy (HAART), which includes nucleoside analogue reverse transcriptase inhibitors, protease inhibitors and/or non-nucleoside reverse transcriptase inhibitors, can slow down the progression to disease but cannot clear the virus
Vaccine	Based on recombinant HBsAg, which induces neutralizing HBsAg-specific antibodies and $CD4^+$ T cell and $CD8^+$ T cell responses	None	None

CCR5, CC-chemokine receptor 5; CXCR4, CXC-chemokine receptor 4; DC-SIGN, dendritic cell-specific ICAM3-grabbing non-integrin (also known as CD209); HBV, hepatitis B virus; HCV, hepatitis C virus; HBsAg, HBVsurface antigen; NA, not applicable; SRB1, scavenger receptor class B member 1.

First, viral receptors or co-receptors may be absent or present only at a low frequency on DCs. DCs express low levels of the principal HIV receptor, CD4, and the co-receptors CC-chemokine receptor 5 (CCR5) and CXC-chemokine receptor 4 (CXCR4)[32] and very low levels of the HCV co-receptor claudin 1 (REF. 28). Unlike HIV and HCV, functional receptors that mediate the entry of HBV have not yet been identified.

Second, the virus may be degraded in intracellular compartments in DCs before it completes its replicative cycle. Antigens can be targeted to different processing pathways after internalization through receptor-mediated endocytosis, and the endocytosed antigen undergoes extensive degradation before its presentation on the cell surface in association with MHC class I and MHC class II molecules[33]. DC-specific ICAM3-grabbing

Box 1 | **Viral strategies for evading the immune response**

As a consequence of their co-evolution with their hosts, viruses have developed various strategies to evade the host immune system and ensure their own replication and survival (reviewed in REF 124).

Antigenic variation
This is an important strategy that is particularly common in RNA viruses. Owing to the low fidelity of viral polymerases, many mutations are introduced into the viral genome during the course of replication, resulting in the existence of many different genetic quasispecies in a single host. This makes it difficult for the host to generate the staggeringly complex immune response that is required for elimination of the virus.

Sequestration
Viruses infect non-permissive or semi-permissive host cells to store their genetic information, thereby becoming invisible to the immune system of the host. These viruses stay 'latent', with absent or decreased transcription, until the virus or cell becomes activated.

Blocking antigen presentation
Collectively, viruses encode proteins with the capacity to interfere with almost any step in antigen processing and presentation by antigen-presenting cells. For example, they can prevent proteasomal antigen fragmentation and antigen transport to the endoplasmic reticulum, interfere with the expression and localization of major histocompatibility complex (MHC) class I and MHC class II molecules or downregulate the expression of co-stimulatory molecules.

Cytokine evasion
Cytokines released by the host in response to viral infections coordinate and control the processes of immune activation and proliferation. It is therefore not surprising that viruses counteract these responses by encoding mimics or homologues of normal cytokines and their receptors that bind to or replace the normal cellular counterparts, thus interfering with cytokine signalling in the host cell.

Inhibition of apoptosis
Apoptosis, or programmed cell death, is a silent and non-inflammatory process that eliminates cells that are infected by a virus. Viruses encode a range of proteins that, between them, block apoptosis at essentially every step to delay cell death until the viral progeny have been formed and are infectious.

non-integrin (DC-SIGN; also known as CD209), a C-type lectin receptor[34], has been shown to promote HIV antigen presentation by MHC class I and MHC class II molecules[35,36]. Scavenger receptor class B member 1 (SRB1) is known to mediate the uptake and presentation of HCV particles by DCs[37].

Third, host factors may block viral replication, or host factors required for replication may be missing in DCs. HBV replication was shown to be dependent on the presence of liver-specific transcription factors belonging to a family of nuclear hormone receptors[38]. Members of a family of cellular restriction factors, the APOBEC (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide) cytidine deaminase family, block replication of HIV after viral entry[39]. Expression levels of APOBEC-like 3G (APOBEC3G) in mDCs correlate with HIV resistance[40], suggesting that cytidine deaminases are potent innate barriers to HIV infection. The presence of host restriction factors might be a crucial factor in determining the susceptibility of DC populations to productive infection with persistent viruses.

The role of dendritic cells in viral dissemination

After uptake of viral antigen, activated DCs can traffic extensively from peripheral tissues to secondary lymphoid organs in an effort to present viral antigens to naive T cells. It is therefore not surprising that persistent viruses exploit this migratory property of DCs to disseminate to more favourable sites of replication.

HIV. It has been known for more than a decade that DCs efficiently transmit HIV to $CD4^+$ T cells. One potential mechanism of HIV transfer from DCs to T cells involves DC-SIGN (reviewed in REF. 16). DC-SIGN-mediated binding of HIV requires the interaction of the HIV envelope glycoprotein gp120 with the carbohydrate recognition domain of DC-SIGN. HIV is subsequently internalized into non-lysosomal compartments and transported within DCs before it is transferred to $CD4^+$ T cells in a process termed *trans*-infection. The sequential endocytosis and exocytosis of intact HIV virions, without viral replication, is called the 'Trojan Horse' model. In this model, virion transmission is thought to occur through the infectious synapse[41] (a structure that is formed between DCs and T cells) and is mediated by viral receptors, co-receptors and DC-SIGN or other C-type lectins. Because DCs can sequester infectious viruses for several days in their endosomal compartments, DCs can carry HIV to interacting T cells in the lymph node, which is the most important site for viral replication and spread[42]. Direct HIV infection of DCs can also occur, although it is less efficient than infection of $CD4^+$ T cells. Several reports indicate that HIV dissemination may be aided by the transfer of progeny virus from infected DCs to T cells[43,44] in a process known as *cis*-infection. It is possible that DCs form a long-lived, motile HIV reservoir that helps to disseminate infectious virus through peripheral blood and lymphoid and non-lymphoid tissues.

The differences between the DC subsets (TABLE 1) raise the possibility that they have distinct roles in HIV transmission. For example, HIV transmission is less efficient in pDCs than in mDCs[45]. In addition, although mDC subsets are known to efficiently transfer HIV to activated $CD4^+$ T cells[16], Langerhans cells seem to prevent HIV transmission by degrading captured HIV particles[46], suggesting that distinct DC subsets can either mediate or prevent HIV transmission.

Box 2 | **Studying the hepatitis C virus–dendritic cell interaction *in vitro***

In contrast to HIV and hepatitis B virus (HBV), studies addressing the interaction of hepatitis C virus (HCV) with dendritic cells (DCs) have been hampered for a long time because of the lack of a robust cell culture system that allows the production of infectious recombinant HCV. Various surrogate models have been used to study the virus–host interaction, such as recombinant viral proteins, which are virus-like particles that are generated by self-assembly of the HCV structural proteins and that closely mimic the properties of native virions. Furthermore, infectious pseudoviruses consist of unmodified HCV envelope proteins assembled onto retroviral or lentiviral core particles and are replication incompetent[125]. They have been used to study HCV entry into target cells. Finally, in 2005 a cell culture system based on the transfection of mRNA from HCV strain JFH1 into a highly permissive human hepatoma cell line became available[126–128]. Until now, studies addressing the interaction of HCV with DCs were limited to the use of recombinant HCV derived from this HCV genotype 2a strain that is highly adapted to a hepatic cell line. Of note, HCV genotype 1 strains are more prevalent and are associated with liver disease in most countries[129]. Although low levels of infectious HCV derived from cell culture have been obtained from an HCV genotype 1a isolate with five adaptive mutations[130], and although progress has been made in the construction of chimeric HCV genomes comprising replicase proteins from HCV strain JFH1 and structural proteins from heterologous HCV strains[131,132], the challenge remains to develop *in vitro* systems for better virus production for other HCV genotypes as well as novel cell culture systems allowing the selection of HCV variants that are particularly adapted to DCs.

HCV. Compared with HIV research, studies analysing the *in vivo* dissemination of hepatotropic viruses by DCs are in their infancy. The HCV envelope glycoprotein E2, HCV virions from serum samples taken from patients infected with HCV, and retroviruses that were pseudotyped with HCV envelope glycoproteins (known as HCV pseudoviruses) have been shown to bind specifically to DC-SIGN[47–49]. Thus, it may be possible that blood DCs or hepatic DCs in the liver sinusoids bind circulating HCV particles through a DC-SIGN-mediated mechanism. Of note, HCV pseudovirus bound to the DC-SIGN that was expressed on monocyte-derived DCs was transmitted efficiently when co-cultured with the human hepatocellular carcinoma cell line Huh7 (a cell line that supports HCV pseudovirus entry and productive viral replication of recombinant infectious HCV)[49,50]. Furthermore, virus-like particles, which are representative of HCV envelope glycoproteins, are bound by DC-SIGN and are targeted to early-endosomal vesicles or non-lysosomal compartments in monocyte-derived DCs. The HCV particles resided in these compartments for over 24 hours[51], suggesting that HCV can bypass the antigen processing and presentation pathways in DCs, thereby escaping degradation. It is possible that HCV retained in the non-lysosomal compartments of DCs plays a part in HCV transmission from DCs to hepatocytes. HCV captured by blood DCs or hepatic DCs in the liver sinusoids may allow transfer of the virus to the underlying hepatocytes when DCs traverse the sinusoidal lumen to the hepatic lymph. Similarly to DCs, liver sinusoidal endothelial cells (LSECs), which form the endothelial lining of the hepatic sinusoid (FIG. 2), have been shown to bind recombinant HCV E2 protein through the interaction of the DC-SIGN and DC-SIGN-related protein (DC-SIGNR; also known as CLEC4M) that are present on the surface of LSECs[52]. However, LSECs cannot support HCV pseudovirus entry and infection with HCV derived from cell culture, suggesting that LSECs are not permissive for HCV infection[52]. Nonetheless, DC-SIGN-mediated binding of HCV to LSECs might support a model whereby this binding provides a mechanism for high-affinity binding of circulating HCV in the liver sinusoid, allowing subsequent transfer of the virus to the underlying hepatocytes and therefore increasing the rate and efficiency of their infection.

HBV. Although DC-SIGN recognizes a broad range of pathogens, from bacteria to viruses, binding to DC-SIGN has not been observed so far for recombinant HBV surface antigen or for HBV particles derived from cell culture[53]. Interestingly, studies have shown that enzymatic modification of the *N*-linked oligosaccharide structures of the HBV antigen prevents recognition by DC-SIGN[53], although other mechanisms might also have a role.

The impact on dendritic cell function

***Effects of persistent viruses on DCs* in vivo.** Virus-mediated impairment of DC function (TABLE 3) is a strategy to attenuate the multiple downstream immune effector mechanisms that depend on optimal DC function, and it might facilitate viral persistence.

At the primary level, viruses can modulate the frequency of DC subsets by interfering with DC development, causing aberrant trafficking or inducing apoptosis. Lower numbers of blood mDCs and pDCs have been observed in patients infected with HIV[54–59], HCV[60–65] and HBV[66] than in uninfected individuals. In HIV infection, DC depletion seems to be due to the migration of pDCs to the inflamed lymph nodes, where they have been found to be activated, apoptotic and frequently infected with virus[67,68], suggesting that HIV-mediated cell death may account for the decreased number of circulating pDCs. In HCV or HBV infection, blood DC subsets are enriched in the liver[64,69–71], suggesting that DC migration to the liver causes the observed paucity of circulating DCs. However, lower numbers of circulating DCs have also been observed in patients with liver diseases that are not related to viral infection, such as granulomatous hepatitis or primary biliary cirrhosis[27,61,72], suggesting that the low DC count in virus-related liver diseases is a common, nonspecific feature of inflammatory hepatitis. Interestingly, *in vitro* studies revealed that HCV envelope glycoprotein E2 and sera from patients infected with HCV inhibit the migration of DCs towards CC-chemokine ligand 21 (CCL21), a CCR7-binding chemokine that is important for homing to lymph nodes[64]. This leads to the hypothesis that after HCV uptake DCs may experience an impaired ability to migrate to the draining lymph node, causing them to be trapped in the liver and, therefore, to be less able to prime T cell responses. However, the *in vivo* relevance of this hypothesis remains to be investigated.

Differentiation and activation of virus-specific T_H1 cells and cytotoxic T lymphocytes from $CD4^+$

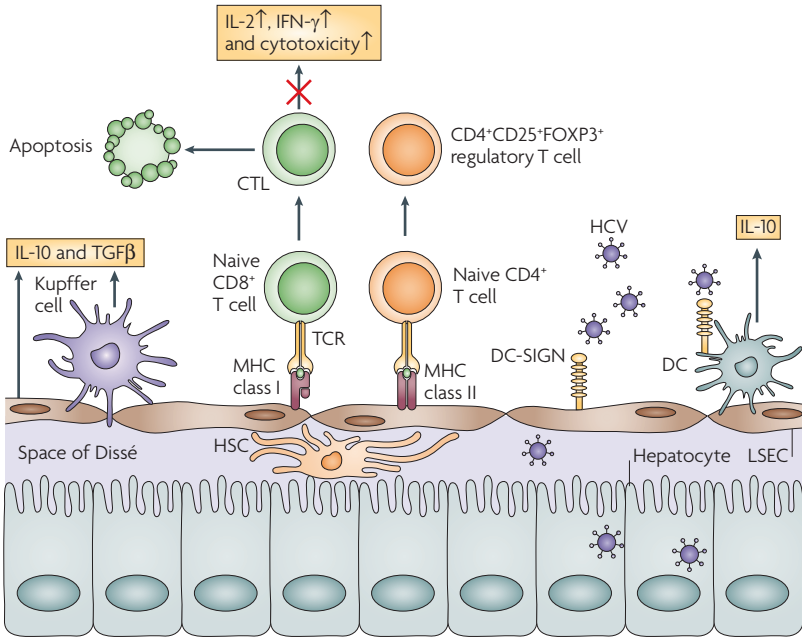

Figure 2 | **Antigen presentation in the liver results in T cell tolerance.** The liver sinusoid is lined by a fenestrated endothelium comprised of liver sinusoidal endothelial cells (LSECs). Kupffer cells and immature dendritic cells (DCs) are found in the sinusoid. Hepatic stellate cells (HSCs) are located in the subendothelial space, known as the space of Dissé. T cells that recognize antigen in the liver (which is presented by major histocompatibility complex (MHC) class I and MHC class II molecules on LSECs and recognized by T cell receptors (TCRs) on T cells) are exposed to immunosuppressive cytokines (namely, interleukin-10 (IL-10) and transforming growth factor-β (TGF-β)) that are continuously synthesized by Kupffer cells, LSECs and DCs. Interaction of naive T cells with antigen-presenting LSECs results in differentiation of T cells into CD4+CD25+FOXP3+ regulatory T cells and impaired cytotoxic T lymphocytes (CTLs) that are unable to produce IL-2 or interferon-γ (IFNγ) or to exhibit cytotoxicity to infected cells and that undergo apoptosis. Hepatotropic viruses seem to be captured by DCs and/or LSECs in a process that probably involves DC-specific ICAM3-grabbing non-integrin (DC-SIGN; also known as CD209) or DC-SIGN-related protein (DC-SIGNR; also known as CLEC4M) for hepatitis C virus (HCV) or other, not-yet-defined cell surface molecules for hepatitis B virus. The viruses are subsequently transferred to the underlying hepatocytes. Viral particles may be internalized by hepatic DCs and LSECs for processing and presentation to naive T cells. Figure is modified, with permission, from *Nature Reviews Immunology* REF. 116 © (2003) Macmillan Publishers Ltd. (all rights reserved) and REF. 133 © (2006) John Wiley and Sons.

T cells and CD8+ T cells, respectively, are regulated by DC-mediated production of IL-12. DC-mediated production of IL-10, on the other hand, is capable of inhibiting these responses[3]. Upregulation of IL-10 production and suppression of IL-12 and IFNα in response to various maturation stimuli have been documented in monocyte-derived DCs, mDCs and pDCs isolated from patients infected with HIV[73–75], HCV[63,65,76,77] and HBV[29]. Furthermore, dysregulated cytokine production by DCs might affect the early antiviral defence mediated by NK cells (FIG. 1). Several lines of evidence indicate that NK cell activity is impaired during HIV infection, in part owing to defective pDC function[78,79]. In particular, defective production of IFNγ by NK cells was attributable to impaired pDC function[79]. Whether the defective production of IFNγ that is seen in NK cells of patients with a chronic HCV infection[80] relies also on impaired crosstalk between DCs and NKs remains to be investigated.

Allogeneic
Describing an organ, tissue or cell from a donor of the same species as the recipient but with different histocompatibility antigens.

DCs isolated from patients infected with HIV[73–75], HCV[63,81–83] and HBV[29] were less able to stimulate T cell activation and proliferation than cells from uninfected individuals, as seen in a mixed lymphocyte reaction. Less efficient allogeneic T cell stimulation by DCs from patients infected with HIV[84] or HCV[82] could be reversed by the neutralization of IL-10, suggesting that virus-induced production of IL-10 by DCs might limit T cell proliferation and activation, skewing the immune response towards tolerance. However, several investigators failed to detect impaired stimulation of allogeneic T cells by DCs isolated from patients infected with HCV[60–62,72,85] and HBV[86]. Possible reasons for these contradictory results are that different experimental and technical settings were used (for example, different isolation protocols or maturation cocktails), that DCs might respond differently depending on their maturation status and uptake of viral antigen, and that the viral load in the infected patients might have varied. In addition, DC dysfunction during infection with hepatotropic viruses may be restricted to the virus-specific response, as the strong global immune dysfunction seen in HIV/AIDS is not observed with HBV and HCV infection.

To gain a different perspective on the impact of persistent viruses on DCs, DC number and function have been studied before and during antiviral therapy. Highly active antiretroviral therapy (HAART) for HIV infection resulted in an increase in pDC number and restoration of IFNα production to normal levels[78,87], indicating that antiretroviral therapy that reduces viral load can reconstitute the function of DCs. Likewise, following therapy with pegylated IFNα and ribavirin for HCV infection, the frequency of pDCs in individuals with viral clearance increased substantially and reached levels observed in uninfected controls[88]. Therapy for HBV infection with the nucleotide analogue adefovir dipivoxil increased the frequency of mDCs and their ability to produce IL-12 as well as the T cell stimulatory capacity, whereas the production of IL-10 decreased[30]. This functional recovery of mDCs coincided with a notable reduction in viral load, underscoring the importance of a reduction in viral load for antiviral regimens; this reduction serves as the first step in a multistep process that culminates in the restoration of impaired immune responses during recovery from persistent viral infections.

In vitro ***studies to investigate the molecular mechanisms of the virus–DC interaction.*** To clarify the impact of persistent viruses on DC function and to identify the molecular mechanisms involved in this interaction, DC subsets isolated from uninfected individuals have been exposed directly to recombinant infectious virus or viral proteins. In contrast to HIV[89] and other viruses, such as influenza virus and HSV1 (REFS 25,90–92), recombinant and serum-derived HCV and HBV have been shown to be poor inducers of IFNα production in pDCs[24,25,65,90], suggesting that these viruses may use this mechanism to downregulate downstream effector functions that are dependent on pDC-mediated IFNα production. In

Table 3 | **Effects of persistent viruses on dendritic cell number and function**

	DC number		Affected DC function				
	In the blood	At the infection site	Production of IFNα	Production of IL-12	Production of IL-10	Induction of IDO activity	T cell stimulation
HCV	Decreased	Enriched (in the liver)	Reduced	Reduced	Increased	Not known	Reduced or normal
HBV	Decreased	Enriched (in the liver)	Reduced	Reduced	Increased	Not known	Reduced or normal
HIV	Decreased	Enriched (in lymphoid tissue)	Reduced	Reduced	Increased	Yes	Reduced
Effect*	• NA	• NA	• Suboptimal T cell and NK cell activation • Reduced plasmacytoid DC and T cell survival	• Suboptimal differentiation of T_H1 cells • Decreased IFNγ production by $CD8^+$ T cell and NK cells	• Inhibition of DC activation • Inhibition of T_H1 type cytokine production • Inhibition of $CD8^+$ T cell function	• Suppression of T cell proliferation and function • T cell apoptosis	• Decreased control of viral replication

DC, dendritic cell; HBV, hepatitis B virus; HCV, hepatitis C virus; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IL, interleukin; NA, not applicable; NK, natural killer; T_H1, T helper 1. *Effect on downstream immune function and disease activity.

pDCs, TLR7 and TLR9 detect viral RNA and DNA, respectively, in endosomal compartments, leading to the activation of nuclear factor-κB (NF-κB) and IFN regulatory factors (IRFs)[4]. IFNα production induced by CpG oligodeoxynucleotides, the TLR9 agonist, but not by resiquimod, the TLR7 agonist, was inhibited by HCV[24,25,90] and HBV[93]. Similarly, HIV inhibited TLR9-mediated IFNα production[59,94], indicating that impairment of IFNα production in pDCs is a strategy that is used universally by persistent viruses. What are the underlying mechanisms responsible for this viral interference in the IFNα pathway of pDCs? A possible mechanism could be related to viral cross-linking of cell surface receptors that downregulate IFNα production, such as the C-type lectins blood DC antigen 2 (BDCA2; also known as CLEC4C) and dendritic cell immunoreceptor (DCIR; also known as CLEC4A). It has been shown that BDCA2 and DCIR ligation and cross-linking results in the inhibition of CpG-mediated induction of IFNα by pDCs[95,96]. The viral envelope proteins HBV surface antigen (HBsAg) and HIV gp120 can directly impair TLR9-mediated IFNα production by pDCs through binding BDCA2 (REFS 93,94). Although TLR9-mediated IFNα production was blocked by HCV core particles[24] and recombinant non-infectious HCV particles composed of HCV core and the envelope glycoproteins E1 and E2 (REFS 24,25,90), it is not known whether the interaction also occurs through BDCA2.

Recent studies indicate that persistent viruses may target immunosuppressive enzymes in DCs to actively suppress antiviral T cell immune responses. The tryptophan-catabolizing indoleamine 2,3-dioxygenase (IDO) activity seems to be a central feature of the suppressive function of DCs. DC-mediated IDO activity has been associated with inhibition of T cell proliferation and function[97]. *In vitro* activated human T cells underwent cell cycle arrest when deprived of tryptophan[98], and T cells became susceptible to apoptosis *in vitro* and *in vivo* in response to the toxic metabolites generated during tryptophan degradation[99]. Direct exposure of DCs to HIV induces IDO activity, leading to the inhibition of $CD4^+$ T cell proliferation *in vitro*[100]. Moreover, HIV-stimulated IDO activity in pDCs induces the differentiation of naive T cells into $CD4^+CD25^+FOXP3^+$ regulatory T cells with suppressive function[101], suggesting that HIV-induced IDO activity may contribute to viral persistence by suppressing virus-specific T cell responses. In simian immunodeficiency virus (SIV)-infected macaques, peak IDO activity coincided with an increase in plasma viraemia and the transient expansion of the regulatory $CD8^+CD25^+FOXP3^+$ T cell subset that may participate in dampening the SIV-specific $CD4^+$ T cell response[102]. As enhanced IDO activity has been observed in patients infected with HIV, HCV and HBV[103–105], the role of DC-mediated IDO activity in viral persistence merits further investigation.

In summary, several lines of evidence indicate that viruses efficiently target DC function to attenuate the antiviral host immune response and establish persistence. But is there also a role for DCs in disease progression? Chronic HBV and HCV infections are major risk factors for the development of hepatocellular carcinoma[14]. There is increasing evidence that a long-standing inflammatory injury is an important procarcinogenic factor in many different cancer types, including hepatocellular carcinoma[106]. The host DC immune response to hepatitis viruses is fairly weak and often fails to control or completely clear infection, resulting in chronic stimulation of the antigen-specific immune response in persistently infected patients. Chronic antigen stimulation at the infection sites and continuous infiltration of DCs into liver tissue may perpetuate a long-lasting chronic inflammatory process owing to the continued expression of pro-inflammatory cytokines, the accompanying activation of liver NK cells and the recruitment of T cells. These events may affect many cellular pathways and ultimately result in fibrosis, cirrhosis or hepatocellular carcinoma. In HIV infection, it is

widely accepted that chronic immune activation has a central role in driving the progression to AIDS[107]. Recent reports indicate that chronic pDC stimulation and IFNα production are associated with a higher risk of progression to AIDS[108,109], underscoring the role of pDCs in the disease. A detailed comparison of the complex processes that govern homeostasis and immune activation mechanisms in health and persistent viral infection may help define the contribution of DCs to disease pathogenesis.

The role of hepatic antigen-presenting cells

The liver has several cell populations that can act as APCs. As well as liver DCs, LSECs, stellate cells and Kupffer cells[110] (FIG. 2) can present antigens and influence the generation and maintenance of the antiviral immune responses. However, the liver-specific immune system is maintained at a baseline state of tolerance, as evidenced by the spontaneous acceptance of liver allografts[111]. Several types of liver APCs exist in a state of active tolerance and contribute to the tolerogenic liver environment by the continuous secretion of immunosuppressive cytokines, for example, IL-10 and transforming growth factor β1 (TGFβ1)[112]. This raises the question of whether the tolerogenic properties of liver APCs contribute to the persistence of hepatotropic viruses and whether the liver is a unique environment for immune evasion.

Owing to the difficulty in gaining access to liver biopsies and the challenge of isolating adequate numbers of APCs from tissue (and of obtaining high-purity samples), limited information is available regarding the role of hepatic APCs in viral infection. In isolated Kupffer cells that were incubated with sera containing HCV RNA (from patients infected with HCV)[113], genomic RNA from HCV disappeared within a few days of infection and the replicative intermediate could not be detected, suggesting that Kupffer cells do not support HCV replication. Similarly, isolated LSECs were unable to support infection by HCV and HBV derived from cell culture, suggesting that LSECs are not permissive for hepatotropic viruses[52,114].

An analysis of liver biopsy samples obtained from patients with chronic HCV infection demonstrated that most Kupffer cells express high levels of co-stimulatory molecules, MHC class I molecules and MHC class II molecules and form clusters with $CD4^+$ T cells, thereby acquiring the phenotype of an effective APC[115]. As Kupffer cells are able to move across the sinusoidal wall into the liver parenchyma, their activation might reflect phagocytosis of HCV-infected apoptotic hepatocytes. Although there is little doubt that liver DCs can take up viral particles, available evidence indicates that this may not translate to efficient T cell priming and activation. *In vivo* studies have revealed that hepatic DCs and LSECs do present exogenous antigen to naive T cells, but the resulting activated T cells either fail to differentiate into effector T cells or acquire an immunosuppressive phenotype (for reviews, see REFS 112,116). It is possible that the uptake of viral particles by liver APCs primes $CD4^+CD25^+FOXP3^+$ regulatory T cells and impairs $CD8^+$ T cells such that they fail to eradicate the virus from the liver. As antigen-specific $CD8^+$ T cells in the liver of patients with chronic HCV infection frequently become dysfunctional and unable to secrete IFNγ or IL-2 (REF. 117), the role of hepatic DCs in HCV-specific T cell priming merits further investigation.

Current research has not focused on the ability of the hepatocyte to act as an APC in HCV infection. In general, hepatocytes are not easily accessible to naive T cells, because LSECs form an effective barrier between hepatocytes and the sinusoidal lumen[118]. However, electron microscopy analysis has shown that hepatocytes have microvilli that project into the sinusoidal lumen through the fenestrations in the endothelium, allowing contact between hepatocytes and circulating T cells in the lumen[119]. Hepatocytes do not normally express MHC class II molecules; however, aberrant expression of MHC class II molecules has been demonstrated in clinical hepatitis[120,121]. It is therefore possible that hepatocytes expressing MHC class II molecules stimulate $CD4^+$ T cells or shape the antiviral immune response of pre-activated $CD4^+$ T cells. Additional studies in transgenic mice showed that $CD8^+$ T cells might be directly activated by hepatocytes. However, this activation seems to favour an impaired cytotoxic T lymphocyte response and reduced host survival, possibly caused by a lack of co-stimulatory molecules[122,123]. Thus, presentation of viral antigens by hepatocytes may influence the antiviral immune response and seems to promote $CD8^+$ T cell dysfunction.

Conclusions and perspectives

Accumulating evidence indicates that HIV, HCV and HBV target DC functions to disturb the generation of strong antiviral innate and adaptive immune responses, facilitating viral persistence. All three viruses seem to use similar strategies to attenuate the potent antiviral IFNα response in pDCs. In addition, these viruses seem to affect the ability of mDCs to produce key cytokines that are essential for the development and activation of an effective T cell response. Not only do these viruses override the natural antiviral activity of the DCs, but they also use the DCs as vehicles for widespread dissemination within the host.

In the future, key issues for improving our understanding of the interplay between persistent viruses and DCs are: the characterization of the intracellular compartments and molecular mechanisms that are required for virus acquisition, processing and presentation by DCs; the identification of mechanisms regulating the balance between intra-hepatic tolerance and immunity; and the role of DCs in aiding viral transmission during infection with HBV and HCV. Not only will knowledge of these mechanisms help us to understand viral pathogenesis, but it can also be used to design strategies that manipulate the immune system towards generating a protective immune response that controls viral replication without the associated immunopathology.

Allograft
The transplantation of an allogeneic organ or tissue or allogeneic cells from a donor to a recipient. Also known as an allogeneic transplant.

Tolerogenic
Non-responsive to a previously encountered antigen.

Sinusoidal wall
A layer of liver sinusoidal endothelial cells that separates the sinusoidal blood (which is derived primarily from the portal vein) from hepatocytes.

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Competing interests statement
The authors declare no competing financial interests.

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HBV | HHV5 | HIV-1 | HIV-2 | HRSV | HSV1
UniProtKB: http://www.uniprot.org
APOBEC3G | BDCA2 | CCL21 | CCR5 | CCR7 | CD4 | CD40 | claudin 1 | CXCR4 | DCIR | DC-SIGN | DC-SIGNR | ICP47 | IFNβ | IFNγ | IL-2 | IL-10 | IL-15 | SRB1 | TGFβ1 | TLR7 | TLR9 | US6

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