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REVIEW

Dissecting the dendritic cell controversy in chronic hepatitis B virus infection

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Therapeutic vaccines to boost endogenous T-cell immunity rely on the stimulatory capacity of dendritic cells (DCs). The functionality of DCs in chronic hepatitis B virus (HBV) infection has been a long-standing debate. Therefore, we have attempted to summarize multiple studies investigating DC function in chronic HBV patients to determine whether common observations can be drawn. We found that the frequency and function of *ex vivo*-tested myeloid and plasmacytoid DCs were largely intact in patients with HBV infection and similar to those of healthy donor DCs. The main exception was reduced IFN-α production by plasmacytoid DC from chronic HBV patients. This reduced IFN-α production correlated with liver inflammation in multiple studies but not with viral load, suggesting that viral antigens have little effect on DC function. The majority of the confusion about DC function arises from studies reporting the reduced function of healthy donor DCs exposed to various sources of HBV *in vitro*. These direct effects of viral antigens are in contrast to data from HBV-infected patients. The variations in the assays used and areas that require further investigation are also covered
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INTRODUCTION

Virus-specific T cells play a central role in the clearance of hepatitis B virus (HBV) infection. Therefore, attempts to develop therapeutic vaccines for chronic HBV infection have focused on inducing or boosting HBV-specific T-cell immunity. However, this goal has proven extremely challenging given the exhausted nature of virus-specific T cells in patients with chronic HBV infection. Virus-specific T cells become progressively more exhausted with increasing viral load and display reduced effector function.[1,2] Years of exposure to viral antigens causes T cells to express an array of inhibitory receptors and makes them highly prone to apoptosis.[3–6] Devising strategies to break this level of immune tolerance or induce new T-cell responses will be critical if vaccine therapy is to be successful.

Multiple attempts have been made to vaccinate patients with chronic HBV infection. Many of the early attempts consisted of using recombinant antigens similar to the prophylactic HBV vaccine. DNA vaccines and immunodominant peptides have also been tested in chronic HBV patients.[7,8] Some of these approaches did increase the HBV-specific T-cell response, but the effect was transient and rarely led to durable responses or to the clearance of HBsAg. Recently, a large Phase II/III clinical trial tested the immunogenicity of HBsAg immunocomplexes because of their ability to activate dendritic cells (DCs); however, the response rates were again limited and were equal to the administration of adjuvant alone.[9] Although these attempts have been largely unsuccessful, they are important in highlighting the level of activation that is required to break T-cell tolerance in chronic HBV infection. Based on the results of these trials, therapeutic vaccines in development have expanded to incorporate new adjuvants[10] and multiple components in combination with antiviral therapy.[11]

T cell-inducing vaccines rely on the network of professional antigen-presenting cells to take up and present vaccine antigens in a context that is capable of reversing T-cell exhaustion. This job primarily falls on dendritic cells, but whether DCs in chronic HBV patients are phenotypically and functionally equal to DCs from healthy donors has been a longstanding point of debate. Data have been presented to support both arguments, ultimately leading to confusion. Because DCs are critical to the efficacy of T-cell vaccines, we have devoted this review to dissecting the different studies on human myeloid

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DCs (mDCs), plasmacytoid DC (pDCs), monocyte-derived DC (moDCs) in HBV infection and DC-based vaccines in an attempt to develop a consensus regarding DC function and phenotype and to identify areas that require additional research. Surprisingly, studies on primary DCs do not show marked ambiguity. Many of the questions arise from studies using moDC cultured *in vitro* or healthy donor DCs/moDCs exposed *in vitro* to HBV antigens or virions. The discussion here is divided according to the type of DCs: primary DCs from patients, moDC from patients and healthy donor DCs exposed to virus or viral antigens *in vitro*. We then address the use of DC-based vaccines and areas where further investigation is needed.

DC OVERVIEW

In the context of HBV research, DC analysis has primarily been limited to those DC populations that can be isolated from the peripheral blood, i.e., mDCs and pDCs. Many of the studies performed on primary DCs have included 3–4 assays, including those that examine DC frequency, phenotype, cytokine production and the ability of DCs to stimulate T-cell proliferation in mixed lymphocyte reactions (MLRs). Each DC subset description below contains a table summarizing the respective DC functions for each study.

mDCs

mDCs, although the dominant DCs in the peripheral blood, are still rare, with a frequency of 0.4%–0.5% of total peripheral blood mononuclear cells (PBMCs). Identification of any DC subset is not a trivial task and requires the detection of multiple surface markers to ensure appropriate identification. Myeloid DC encompass two subsets in the blood: CD1c (BDCA-1) and CD141 DC (BDCA-3). In practice, they are identified as lineage negative (CD3, CD14, CD16, CD19, CD20, CD56-negative), HLA-DR$^+$and CD11c$^+$/CD1c$^+$ or CD11c$^+$/CD141$^+$. mDC seed tissues, efficiently internalize antigen, produce T-cell polarizing cytokines and process and present antigen to T cells.

The majority of studies have found that the frequency of mDCs is unchanged in chronic HBV patients compared with healthy donors (Table 1). This was true for adult patients and for both pediatric patients[12] and infants[13] born to HBV-positive mothers. A similar observation was made in terms of mDC cytokine profiles, in which the IL-12p70 and IL-10 production was equal between HBV patients and healthy donors.[12,14–17] Exceptions are present, however. Zhang *et al.*[12] reported a lower frequency of mDCs in the blood of immune active pediatric patients, but not in those who were immune-tolerant. A study by van der Molen *et al.*[18] found that mDC frequencies were reduced in chronic HBV patients. However, mDC frequency could be returned to the level of healthy donors with antiviral therapy. In addition, IL12p70 production increased and IL-10 production decreased as a result of adefovir treatment, suggesting that antiviral therapy can normalize functional aberrations.

The phenotype of mDCs has not been extensively studied, but the costimulatory molecule expression appears to be similar to that of healthy mDC that were directly examined *ex vivo*. van der Molen *et al.* observed differences in the frequency of CD80$^+$ and CD86$^+$ mDCs after *in vitro* maturation, but these differences were small (CD80, 74% HBV *vs.* 84% healthy; CD86, 81% HBV *vs.* 92% healthy) and not related to viral load or ALT.[15] Another deviation from healthy donor mDC patterns was reported by Chen *et al.*,[19] who found that the frequency of PD-L1$^+$ mDC was significantly increased in patients with active hepatitis. Less than 1% of mDCs expressed the inhibitory PD-L1$^+$ molecule in healthy donors, whereas patients with active hepatitis had a frequency of approximately 7% PD-L1$^+$ mDCs. The frequency of PD-L1$^+$ mDCs positively correlated with both viral load and ALT. However, PD-L1 expression is likely more closely linked to ALT than viral load because the immunotolerant patients (high viral load, normal ALT) in the same study had a significantly lower frequency of PD-L1$^+$ mDC compared with the patients with active hepatitis.

The assay demonstrating the most variability between studies is T-cell proliferation using mixed lymphocyte reactions. This assay also inherently incorporates the most variability because it relies on the activation of allogeneic T cells due to mismatches that occur primarily at the MHC II locus. The studies listed in Table 1 are nearly evenly divided as to whether the ability of mDCs to stimulate T-cell proliferation in MLR assays is impaired. Therefore, this question remains unanswered, but we discuss points of variability between the assays and potential solutions and thereby try to limit this confusion.

pDCs

pDCs represent an even smaller fraction of the PBMCs, with an average frequency of 0.2%–0.3% of total leukocytes. They are identified by the expression of CD303 (BDCA-2) or CD123 on lineage negative (CD3, CD14, CD16, CD19, CD20, CD56-negative), CD11c$^-$, HLA-DR$^+$ cells. pDCs can present antigen to T cells but are the primary producers of IFN-α. Therefore, they have the potential to influence the nature of innate immune responses during infection and are a primary target of new synthetic Toll-like receptor(TLR)-based antivirals.[20]

Similar to mDCs, the majority of studies have found that the frequency of pDCs was similar between chronic HBV patients and healthy donors (Table 2).[14–17,21–23] As noted above for mDCs, this result was consistent for adults, pediatric patients[12] and infants born to HBV-positive mothers.[13,24] Again, some exceptions exist. Pediatric patients with immune active disease or infants born to mothers with $>5\times10^7$ copies/ml HBV also showed a reduced pDC frequency in the blood.[12,24] In two studies where reduced frequencies were observed in adult patients, the pDC numbers recovered with antiviral therapy,[18,25] again suggesting that DC frequency can be restored with effective treatment.

The phenotype of pDCs has been similar between HBV patients and healthy donors in terms of costimulatory molecule expression, i.e., CD40, CD80 and CD86. One study by Martinet *et al.*[22] showed that the expression of CD40 and CD86 was increased in pDCs from HBV patients; however, the reduced expression of the OX40 ligand in highly viremic patients was also observed.[22] In terms of their ability to stimulate T-cell

Table 1 Summary of data from studies analyzing primary myeloid DCs

Author	*Patients*	*Maturation stimuli*	*Frequency*	*Phenotype*	*Cytokine stimulus*	*Cytokine response*	*Allo T cells*	*MLR response*	*Antigen-specific response*	*Notes*
Beckebaum[14]	12 mixed patients±cirrhosis	NA	=	NT	NA	NT	NT	NT	NT	
van der Molen[15]	Treatment naive, 15 $HBeAg^+$, 15 $HBeAg^-$, ±fibrosis	IL-1β and TNF-α	=	1. =*ex vivo* HLA-DR/CD80/CD86 2. <% CD80/86 after maturation	polyI:C+IFN-γ	1. =IL-12p70, IL-10, IL-6 2. <TNF-α	Total T cells	<	NT	1. TNF-α increased with high viral load 2. Phenotype/MLR response not related to viral load or ALT
van der Molen[18]	12 mixed patients, ±cirrhosis Adefovir treatment	IL-1β and TNF-α	<, restored with therapy	No change with therapy	polyI:C+IFN-γ	1. IL-12p70 increased with therapy 2. IL-10 decreased with therapy 3. TNF-α no change	Total T cells	Increased with therapy	NT	No correlation for IL-10 or IL-12 production and HBV DNA or ALT
Tavakoli[16]	38 patients High viral load and ALT 38 patients low viral load & ALT—no cirrhosis	IL-1β, TNF-α, IL-6, PGE2	=	=	1. CD40Ligand 2. *S. aureus* Cowan strain 1 3. CpG2006 4. polyI:C	=	CD4 T cells	=	=peptide-specific IFN-γ production with T-cell clone	1. longitudinal frequency and phenotype analysis show variations
Chen[19]	Immune active, Immune-tolerant, healthy	polyI:C	NT	>PD-L1	NA	NT	CD4 or CD8 T cells	<, restored by blocking PD-L1	NT	
Gehring[17]	Mixed	NT	=	NT	NA	NT	Total T cells	=	NT	
Zhang[12]	Pediatric Immune-tolerant Immune-active	NT	=Tolerant <Active	NT	polyI:C	=IL-12 in all groups	NT	NT	NT	
Koumbi[13]	Cord blood Neonates	NT	=	NT	NT	NT	NT	NT	NT	

Abbreviations: DC, dendritic cell; HBV, hepatitis B virus; MLR, mixed lymphocyte reaction.
<, lower than healthy; >, higher than healthy; =, equal to healthy; NT, not tested; NA, not applicable.

Table 2 Summary of data from studies analyzing primary pDCs

Author	*Patients*	*Maturation stimuli*	*Frequency*	*Phenotype*	*Cytokine stimulus*	*Cytokine response*	*Allo T cells*	*MLR response*	*Notes*
Beckebaum[14]	12 mixed patients ±cirrhosis	N/A	>	NT	NA	NA	NA	NT	
Duan[25]	80 chronic active hepatitis HBeAg$^+$ 27 cirrhosis	NA	<, partial recovery with therapy	NT	HSV-1	1. <IFN-α 2. Therapy restored IFN-α production	NA	NT	1. PBMCs used in IFN-α production experiments—no pure pDCs. 2. Restored IFN-α in patients where frequency recovered
van der Molen[15]	Treatment naive, 15 HBeAg$^+$, 15 HBeAg$^-$, minimal fibrosis	IL-1β and TNF-α	=	=	*S. aureus* Cowan strain I	1. <IFN-α 2. =IL-10, IL-6 and TNF-α	nylon wool purified total T cells	=	1. IFN-α reduced with high ALT 2. No correlation in IFN-α production with viral load
van der Molen[18]	12 mixed patients, ±cirrhosis Adefovir treatment	IL-1β and TNF-α	<, partial recovery with therapy	NT	*S. aureus* Cowan strain I	1. IFN-α or IL-10 no change with therapy 2. Reduced TNF-α with therapy	NA	NT	
Tavakoli[16]	38 patients High viral load and ALT 38 patients Low viral load and ALT—no cirrhosis	IL-1β, TNF-α, IL-6, PGE2	=	=	1. CD40L 2. *S. aureus* Cowan strain I 3. CpG2006 4. polyI:C	=	CD4 T cells	=	1. Longitudinal frequency/phenotype analysis show variations
Woltman[21]	15 HBeAg$^+$ 10 HBeAg$^-$	NA	NT	NT	CpG	<IFN-α	NA	NT	1. IFN-α reduced with high ALT 2. No correlation in IFN-α production with viral load
Martinet[22]	118 aviremic 67 viremic	NA	=	>CD40 and CD86 <OX40L in viremic patients	1. CpG2336 2. Inactivated flu virus	1. <IFN-α and IP-10 2.<IL-6 and TNF-α in aviremic patients	NA	NT	1. IFN-α production not related to viral load 2. Reduced activation of NK cytotoxicity
Shi[23]	30 treatment naive	NA	<	NT	CpG2216	<IFN-α	NA	NT	
Gehring[17]	Mixed	NA	=	NT	NA	NT	NA	NT	
Zhang[12]	Pediatric Immune-tolerant Immune-active	NT	=Tolerant <Active	NT	CpG2216	<IFN-α in immune-active	NT	NT	IFN-α production equal to healthy in Immune tolerant
Koumbi[13]	Cord blood Neonates	R848	=	=	R848	=IFN-α	NT	NT	TLR-7-mediated IFN-α

Abbreviations: MLR, mixed lymphocyte reaction; pDC, plasmacytoid dendritic cell; TLR, Toll-like receptor.
<, lower than healthy; >, higher than healthy; =, equal to healthy; NT, not tested; NA, not applicable.

proliferation, pDCs from chronic HBV patients were as efficient as those from healthy donors in the two studies that investigated this function.[15,16] Although small discrepancies were present, the frequency, phenotype and ability of pDCs from chronic HBV patients to stimulate T-cell proliferation do not generally differ from healthy donors.

Conversely, cytokine production by pDCs did show significant differences between chronic HBV patients and healthy donors. A majority of studies showed that TLR-9 mediated IFN-α production by pDCs is reduced in chronic HBV patients compared with healthy pDCs.[15,21–23,25] The mechanism of reduced IFN-α production remains to be determined. The downregulation of TLR-9[26] and interference with the mTOR pathway[21] have been suggested as potential mechanisms, but these studies were performed on healthy donor pDCs exposed to HBV *in vitro*. It seems unlikely that this effect would be directly modulated by the virus or viral antigens because four studies found no correlation between the viral load in patients and reduced IFN-α production by pDCs.[15,16,21,22] Two studies have stated that IFN-α production by pDCs negatively correlated with ALT.[15,21] Therefore, regulatory mechanisms to control inflammation during chronic viral infection may alter the ability of pDCs to produce IFN-α.

moDCs and *in vitro* exposure to HBV antigens

moDC generation involves the *in vitro* differentiation of either adherent cells isolated from the PBMCs or purified $CD14^+$ monocytes ($CD14^+$ MN). These cells are believed to mimic the inflammatory DC population that differentiates during periods of inflammation and is capable of promoting Th1-polarized T-cell development through IL-12p70 production and antigen processing. The standard approach used to differentiate monocytes to moDCs involves 5–6 days of *in vitro* culture with GM-CSF and IL-4, followed by an activation cocktail to induce maturation. Inducing maturation increases antigen processing and upregulates costimulatory molecules on the surface of moDCs.

$CD14^+$ MN (monocyte) circulating in patients internalize and retain a depot of HBV antigens.[17] This seems to have little impact on their ability to respond to inflammatory stimuli[27] or induce T-cell proliferation,[17] suggesting that the monocyte, i.e., the moDC precursor, function is intact. There have only been a few studies in which monocytes were isolated from chronic HBV patients, differentiated to moDCs and compared with healthy moDCs (Table 3). A conclusion shared in these studies suggests that the expression of costimulatory molecules was lower on immature moDC[14,28–30] from HBV-infected individuals than from healthy individuals; however, the expression recovered to the level of healthy donor moDCs following maturation. There is mixed information in the studies regarding the analysis of cytokine production and proliferation stimulation by moDCs in MLR reactions.

In addition to moDCs, primary DC populations from healthy donors have been used in experiments to model HBV exposure *in vitro*. We believe that this is where most of the controversy about DC function in chronic HBV infection arises. Experiments have been performed on moDCs, mDCs and pDCs (Table 4). All of the studies report a reduction in costimulatory molecule expression following HBV exposure.[21,23,31,32] However, different antigens or virions regulated the expression of different receptors. Similarly, most studies have found cytokine production to be impaired following exposure to HBV or HBV antigens; however, similar to the phenotype experiments, different forms of the antigen had different effects (Table 4).

These experiments use a variety of antigens, including those isolated from patient serum, recombinant antigens derived from *Escherichia coli* or Chinese hamster ovary (CHO) cells, virus isolated from patient serum or virus isolated from HepG2.2.15 cell supernatant. The variability in source material for these experiments raises significant questions regarding the comparability of their results, as studies have demonstrated that the source of HBsAg can alter how they are bound and internalized.[33–36] Most of the studies have tried to control for

Table 3 Summary of data from studies analyzing monocyte-derived DCs

Author	*Patients*	*Maturation stimuli*	*Phenotype*	*Cytokine stimulus*	*Cytokine response*	*Allo T cells*	*MLR response*	*Antigen-specific response*
Beckebaum[14]	12 mixed patients±cirrhosis	TNF-α, IL-1β, IL-6, PGE2	1. <% CD80/86 2. Phenotype restored upon activation	TNF-α, IL-1β, IL-6, PGE2	<IL-12p70	Naive T cells	<	
Tavakoli[29]	18 active HBV 17 inactive carriers	TNF-α, IL-1β, IL-6, PGE2	1. <CD86, CD40 HLA-DR 2. Phenotype restored upon activation	LPS, HBcAg	=IL-12p70 and TNF-a	CD4 T cells	=	=HBcAg-specific CD4 T-cell clone activation
Zheng[30]	15 $HBeAg^+$ 15 $HBeAg^-$	TNF-α	=	HBsAg	=IL-12p40	NA	NT	=Tetanus toxoid proliferation
Duan[28]	12 mixed patients	TNF-α, IL-1β, IL-6, PGE2	1. <% CD80/86 2. Phenotype restored upon activation	NA	NT	PBMCs	<	

Abbreviations: DC, dendritic cell; HBV, hepatitis B virus; MLR, mixed lymphocyte reaction.
<, lower than healthy; >, higher than healthy; =, equal to healthy; NT, not tested; NA, not applicable.

Table 4 Summary of data from studies analyzing from *in vitro*-treated DCs

Author	*Patients*	*Antigen source (control)*	*Maturation stimuli*	*Phenotype*	*Cytokine stimulus*	*Cytokine response*	*Allo T cells*	*MLR response*	*Antigen-specific response*
Beckebaum[31]	Healthy moDCs	Serum HBV	TNF-α, IL-1β, IL-6, PGE2	1. CD80 lower in HBV-treated	TNF-α, IL-1β, IL-6, PGE2	IL-12p70 not changed	PBMC	Reduced by HBV	Tetanus proliferation reduced by HBV
Op den Brouw[32]	Healthy mDCs	1. Pepsin-treated HBsAg from sera 2. rHBsAg from CHO (CHO cell lysate as ctrl) 3. HBV from HepG2.2.15 (regular HepG2 supernatant)	TNF-α, IL-1β	1. rHBsAg inhibited CD86 upregulation 2. Serum HBsAg inhibited CD40 and CD86 upregulation 3. HBV inhibited CD40 and CD80 upregulation	PolyI:C+IFN-γ	1. HBV inhibited IL-12p70 2. No effect with HBsAg	Total T cells	MLR reduced by serum HBsAg, rHBsAg and HBV	NT
Woltman[21]	Healthy pDC	1. HBV from HepG2.2.15 (regular HepG2 supernatant) 2. Patient HBV (healthy serum) 3. CHO HBsAg and HBeAg 4. *E. coli* HBcAg	CpG2336, herpes simplex virus, loxoribine	HBV impaired upregulation of CD40 and CD86 by CpG	CpG	1. IFN-α reduced by HBV, HBsAg and HBeAg 2. HBcAg had no effect	NA	NT	NT
Shi[23]	Healthy pDC	1. Formaldehyde-inactivated serum HBsAg 2. CHO rHBsAg	NA	NT	CpG2216	1. Fixed serum HBsAg inhibited IFN-α production. 2. No effect of CHO HBsAg	NA	NT	NT

Abbreviations: DC, dendritic cell; HBV, hepatitis B virus; mDC, human myeloid DC; MLR, mixed lymphocyte reaction; moDC, monocyte-derived DC; pDC, plasmacytoid DC.
<, lower than untreated; >, higher than untreated; =, equal to untreated; NT, not tested; NA, not applicable.

sample variation to some extent, but it is not clear whether extracting healthy donor serum or culture supernatant from a different HepG2 cell line is the same as patient serum or culture supernatant from HepG2.2.15 cells minus HBV proteins. None of the studies used an irrelevant antigen purified from CHO cells as control. In addition, multiple studies on primary DCs from chronic HBV patients concluded that viral load had little influence on mDC and pDC function.[15,16,21,22] Instead, the studies suggested that increased ALT levels correlated with impaired IFN-α production by pDCs[15,21]and increased PD-L1 expression on mDCs.[19] Therefore, it is the pathology associated with HBV infection, rather than the virus itself, that likely affects DC function, which cannot be modeled *in vitro* by the addition of antigens. Despite this possibility, neither DC function nor phenotype has been tested as a function of HBsAg concentration in the serum, which can vary significantly with viral load.

POINTS OF VARIABILITY

Although many of the assays performed in the studies reviewed here fall into common categories, there were significant variations in how they were performed. The first obvious point of variability is the patient population. This variability is often inherent in HBV research due to the highly heterogeneous nature of chronic HBV infection. Some studies used well-controlled groups of patients, but these were generally categorized by viral load or HBsAg status. ALT is considered a secondary parameter, and HBsAg quantification has only recently been used to stratify patients. Another confounding variable is the incidence of fibrosis and cirrhosis, which may alter immune responses and is not always known for patient cohorts.

In addition to patient variability, the cocktails used to mature DCs and stimulate cytokine production *in vitro* differed across studies. Some studies used defined synthetic TLR agonists to stimulate cytokine production, whereas others used

whole bacteria or viruses. How much this affects the cytokine profile is unclear, but this difference makes direct comparisons between different studies challenging. Another point of assay variability were the allogeneic cells used for MLR reactions. These ranged from naive T cells to total PBMCs, which compounds the high levels of variation due to the dependence on HLA mismatch between cells. Additionally, most of the assays were measured as raw counts per minute using tritiated thymidine assays, rather than being normalized and analyzed using a stimulation or proliferation index.

POTENTIAL OF MODC VACCINES

Whereas the studies above, which investigated the general properties of moDCs from chronic HBV patients, reported somewhat conflicting data, the use of moDCs as a vaccine has demonstrated relatively positive results. Two studies demonstrated that MHC-II antigen processing and presentation by moDC from chronic HBV patients is intact and capable of stimulating CD4 T cell proliferation similar to moDC from healthy donors.[29,30] We demonstrated that $HBsAg^+$ CD14 monocytes, when differentiated to moDCs, cross-presented *in vivo*-captured antigen and expanded autologous HBV-specific CD4 and CD8 T cells.[17] Similarly, other studies have shown that autologous moDCs loaded with recombinant HBV antigens were capable of stimulating autologous T-cell proliferation.[37–39]

Because of their stimulatory capacity, moDCs loaded with recombinant antigens or synthetic peptides have been administered to patients in two clinical trials. In both cases, a significant improvement in virological parameters was observed in approximately 50% of the patients, with the HBeAg-negative patients responding best.[40,41] The caveat of these trials is the lack of immunological data reported. Only virological and clinical parameters were measured, and the HBV-specific T-cell response was not analyzed. However, the positive response rates suggest that the stimulatory nature of moDCs may have an effect on HBV immunity, but much more work is necessary.

AREAS OF FURTHER INVESTIGATION

Continuing to perform studies using basic methods of cytokine production analysis or MLR stimulation of DCs from HBV patients is unlikely to resolve any of the outstanding issues noted here. More specific assays need to be employed to test the defined functionality of DCs. The cytokine profile of circulating primary mDCs in chronic HBV patients suggests they can stimulate the Th1 responses that are associated with HBV control. However, their ability to process and present antigens to expand virus-specific T cells has not been widely studied. Antigen processing and presentation by mDCs has been reviewed recently,[42] but testing this is not a simple task because of HLA restriction and the low frequency of mDCs in the peripheral blood.

The context in which DCs present antigen can lead to immunity or tolerance.[43] We have shown that DCs and monocytes in the peripheral blood do not constitutively present circulating antigen to virus-specific CD8 T cells,[17] but little is known about presentation to CD4 T cells or antigen presentation by liver or splenic DCs in chronic HBV patients. Studying antigen processing in primary mDCs from chronic HBV patients will be important to assess their potential for activating virus-specific T cells.

Some of this information has been generated in mouse studies. HBV, HBV subviral particles and HBV antigens are processed and presented by mouse monocytes and DCs.[44–46] Shimizu *et al.*[47] demonstrated that primary splenic DCs from HBV-transgenic mice could present *in vivo* captured antigen to T cells. This information is most important in the context of immunomodulatory therapy for chronic HBV. The injection of CD40 ligand into HBV-transgenic mice was found to shift the T-cell response from tolerance to Th1 mediated immunity.[48] Furthermore, CpG administration to mice with chronic HBV infection was shown to induce local intrahepatic T-cell proliferation, contributing to disease resolution.[49] Understanding these mechanisms and how they affect antigen presentation and virus-specific T-cell activation is particularly important, given the move toward developing synthetic oral TLR agonists for immunomodulatory therapy.

In addition to antigen processing, a clear area requiring further investigation is the human intrahepatic immune environment. DCs and monocytes in the blood are considered immature and differentiate when they enter tissues. We and others have characterized the cell populations flushed from the sinusoids of the healthy liver and showed significant variation in cellular content and cytokine profiles compared with PBMCs.[50–52] However, almost no information is available to compare the differences in these populations between HBV patients and healthy donors. Analyses of HBV-infected livers are often restricted to biopsy-sized samples or are explants due to advanced disease; as such, the processes required to extract cells are quite harsh, possibly interfering with or inhibiting downstream analysis. In addition, fibrosis and cirrhosis are likely to have a significant impact on DC function in the liver.

Murine studies have provided a solid foundation from which to characterize the intrahepatic immune environment.[53,54] The liver can induce tolerogenic DCs from hematopoietic progenitors.[55] DCs in the mouse liver produce more IL-10 and display reduced T-cell stimulatory capacity compared with splenic DCs.[56,57] The little information that is known about human intrahepatic DC function corroborates these general observations.[58,59] Despite the challenges of accessing specimens and extracting cells, a better understanding of how DCs and monocytes function in the intrahepatic environment is important for therapeutic development.

CONCLUDING REMARKS

In studies performed on primary DCs that have been tested directly *ex vivo*, mDC and pDC dysfunction was minimal—their frequency, phenotype and cytokine production were similar to those observed for healthy donors. Importantly, the primary DCs do not display an overtly regulatory phenotype. PD-L1 on mDCs was moderately elevated in patients with active disease, and the IFN-α production by pDCs was reduced

compared to healthy donors. However, these findings correlated with the ALT levels and not with viral load, suggesting associations with disease pathogenesis, which can be reduced with antiviral therapy. This is in line with recent therapeutic vaccination attempts in chronically infected woodchucks, in which antiviral therapy promoted T-cell responses to a DNA prime/adenovirus boost vaccine regimen.[11] Therefore, appropriately adjuvanted vaccines have the potential to effectively activate DCs in chronic HBV infection and stimulate T-cell immunity.

There is a considerable lack of knowledge regarding the ability of DCs from HBV patients to process and present viral antigens. Data in this area would help resolve questions related to the mixed data using MLR assays and provide strategies to optimize DC activation for therapeutic vaccination. Although many of the studies using healthy donor DCs treated with HBV antigens *in vitro* were carefully performed, these data should be assessed carefully. In some studies, exposure to different forms of HBsAg resulted in conflicting results, suggesting that variations in antigen preparation can affect assay outcomes. More mechanistic insight into these pathways and confirmation using clinical material will help resolve these controversies.

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