REVIEW

Dendritic cells and immune regulation in the liver

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Hepatic dendritic cells (DC) unquestionably play important roles in the induction and regulation of immune responses. Due to their paucity, functional characterisation of these important antigen presenting cells has been slow but use of DC growth factors (in particular GM-CSF and Flt3L) that markedly enhance their numbers has proved helpful in furnishing adequate study material. While there is growing evidence that DC function is affected in the pathogenesis of liver disease, most work to date has been performed on non-hepatic DC. Increasing knowledge of hepatic DC biology is likely to improve our understanding of disease pathogenesis and resistance to and therapy of liver disease.

INTRODUCTION

The liver is an important site of infectious, parasitic, autoimmune, and malignant diseases. Immune responses and their modulation within the liver are critical to the outcome of these conditions and also in liver transplantation. The inherent tolerogenicity of the liver, including its possible role in oral tolerance, poses important questions about how immune reactivity in the liver is regulated. Increasing attention has focused on antigen presenting cells (APC) and the critical roles that they play in both innate and adaptive immunity. APC exist in several forms within the liver and exhibit a spectrum of abilities to capture, process, and present antigen (Ag) to immune effector cells. Although rare, dendritic cells (DC) are the most highly specialised APC, with ability both to instigate and regulate immune reactivity. In addition, DC are well equipped to migrate from peripheral tissue sites such as the liver to regional lymphoid organs, where they present Ag to T cells. In the normal steady state, these events may be important in the maintenance of self tolerance. It is now recognised that the microenvironment in which APC develop or are activated influences their function and their effects on T cell populations. Furthermore, different DC subsets have been identified that exhibit distinct functional capabilities. Progress in uncovering the properties of liver DC has been slow but the recent surge of interest in DC biology and technological advances in their isolation and characterisation have brought these cells to centre stage in the quest for a fuller understanding of immune regulation within and beyond the liver.

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LIVER APC POPULATIONS COMPARED

The liver contains several types of APC (fig 1). Liver sinusoidal endothelial cells (LSEC) line the sinusoids and have a distinct morphology in comparison with vascular endothelial cells that line arterial branches, and central and portal veins.[1 2] In contrast with vascular endothelia, LSEC do not express CD31 (PECAM-1, pgIIa endothelial cell adhesion molecule), which is expressed at tight junctions of vascular endothelia, but exhibit higher constitutive levels of CD54 (ICAM-1, intercellular adhesion molecule 1) and CD106 (VCAM-1, vascular cell adhesion molecule).[1 2] Also, LSEC have fenestrations up to 100 nm in diameter although passage of particles through these openings is selective. Thus although particles as small as 15 nm fail to enter, lymphocytes can access the space of Disse between the lumen of the sinusoids and hepatocytes. Extracellular matrix and hepatic stellate cells are located in this area. Hepatocytes have been reported to act as APC in certain situations although they are not considered to be primary mediators in immune regulation within the liver.[1 3] Kupffer cells (KC), the resident macrophages of the liver, patrol the portal venous system via the sinusoidal lumen and can adhere to LSEC, occasionally causing temporary obstruction of blood flow through the sinusoid (fig 1).[4 5] In normal liver, hepatic DC typically reside only around portal triads[6–8] and, like DC in other peripheral sites, are able to efficiently capture, process, and transport Ag to regional lymphoid tissues. All three APC (LSEC, KC, DC) internalise Ag by phagocytosis, receptor mediated endocytosis, or pinocytosis but their phenotypes differ considerably.[1 2 9] LSEC and KC express major histocompatibility complex (MHC) Ags, costimulatory and adhesion molecules, and make interleukin (IL)-1 and interferon γ (IFN-γ), suggesting that these cells are at a relatively mature stage.[1 2 9 10] Freshly isolated hepatic DC on the other hand are predominantly immature cells, expressing surface MHC but few costimulatory

Abbreviations: Ag, antigen; APC, antigen presenting cells; BM, bone marrow; CC and CXC, chemokines; CCR and CXCR, chemokine receptors; DC, dendritic cell; ECM, extracellular matrix; Flt3L, fms-like tyrosine kinase 3 ligand; GM-CSF, granulocyte macrophage-colony stimulating factor; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; KC, Kupffer cell; IL, interleukin; IFN-γ, interferon γ; LSEC, liver sinusoidal endothelial cells; MHC, major histocompatibility complex; NPC, non-parenchymal cells; PALT, portal tract associated lymphoid tissue; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; TGF-β, transforming growth factor β; TNF-α, tumour necrosis factor α.

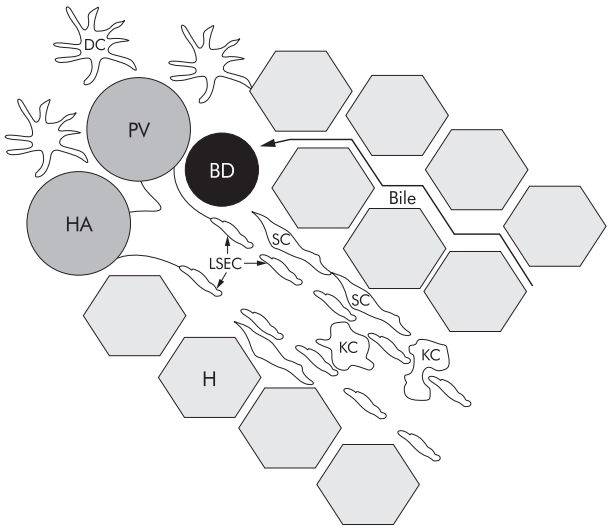

Figure 1 Anatomy of sinusoids. The area between the LSEC and hepatocytes, where extracellular matrix and stellate cells reside, is called the space of Disse. Kupffer cells and other immune cells are believed to extravasate through the LSEC fenestrations into the parenchyma. DC normally reside only in the portal areas. BD, bile duct; DC, dendritic cell; H, hepatocyte; HA, hepatic artery; KC, Kupffer cell; LSEC, liver sinusoidal endothelial cell; PV, portal vein; SC, stellate cell.

molecules necessary for T cell activation.[11–13] Compared with more mature bone marrow (BM) derived or spleen DC, they stimulate naïve allogeneic T cells only poorly.[13–15]

ROLE OF THE LIVER MICROENVIRONMENT AND HEPATIC DC IN TOLEROGENICITY

The immature phenotype of resident hepatic DC coupled with the inherently unique liver microenvironment potentially makes these APC different from DC in other tissue sites (that is, BM, spleen). Although not considered to be an immune privileged site, such as the anterior chamber of the eye or the testis, there are marked similarities between the cytokine milieu of the liver and these other sites. KC and LSEC constitutively express the anti-inflammatory cytokines IL-10 and transforming growth factor β (TGF-β) that are upregulated on stress, while hepatocytes secrete IL-10 in response to autocrine and paracrine TGF-β.[1 2 16 17] Lipocytes, another liver specific cell population that includes Ito and stellate cells, also express increased TGF-β on activation or stress.[16] These cytokines not only affect T helper (Th) T cell differentiation directly (skew to Th2) but also can confer tolerogenicity on DC and other APC by inhibiting their maturation and T cell stimulatory function.

> "There is now much evidence that DC can be rendered tolerogenic"

Although mature DC, rich in surface MHC and costimulatory molecules, are potent stimulators of immune (T cell) function, there is now much evidence that DC can be rendered tolerogenic. Thus exposure of replicating DC progenitors to IL-10 or TGF-β[18] generates DC that are suppressive or tolerogenic. Steinbrink and colleagues[19] showed that culture of immature blood derived human DC with IL-10 inhibited their maturation. Similar results have been obtained with DC transduced with either IL-10 or TGF-β.[20 21] Lack of adequate costimulatory molecule expression, either due to immaturity or exposure to costimulatory pathway blocking agents, can also result in tolerogenic DC, as shown in both allograft[22] and autoimmune disease[23] models.

PHENOTYPE OF HEPATIC DC

Many different markers have been used to identify rodent and human DC, including those that are species specific (table 1). While none are specific to hepatic DC, variations occur in the level of expression of certain markers compared with others. CD11c is a common but not universal marker for DC detection in the murine system. In addition, other markers, such as CD205, have been used by different groups to identify specific murine DC subsets. The two principal subsets identified in mouse liver as well as in lymphoid tissue are the "so-called" myeloid ($CD8\alpha^-CD11b^+$) and lymphoid related ($CD8\alpha^+CD11b^-$) subsets of DC. These DC are distinguished by their reciprocal expression of CD8α and CD11b and were thought initially to have distinct lineage and functions.[24 25] Recent evidence has shown that these subsets derive from a common precursor and that rigid lineage affiliations between subsets may not exist.[26–28] Plasmacytoid DC or type 1 IFN producing cells (a unique cell type of the haematopoietic system) have recently been identified in mouse lymphoid tissues.[29–31] These DC are $CD11c^+CD11b^-CD19^-B220^+$ and $Gr1^+$ and may play crucial roles in antiviral immunity. Whether they are present in normal liver has yet to be determined.

DC have been generated in vitro from mouse liver stem/progenitor cells in response to granulocyte macrophage-colony stimulating factor (GM-CSF). These liver derived DC progenitors[32 33] are distinct in phenotype from DC freshly isolated from normal liver and are $CD11c^{hi}CD24^+CD44^+$. Maturation of DC is associated with upregulation of MHC II, CD80, and CD86, with CD205 being an additional marker used by some groups. Lu and colleagues[34] have also shown that culture of normal murine hepatic non-parenchymal cells (NPC) with IL-3 and CD40L yields a unique population of DC-like cells that are $CD205^{hi}CD11c^-B220^+CD19^-$.

Less diversity has been reported to date for DC markers in the rat and human. OX62, an integrin molecule, is commonly used to detect rat DC.[35–37] As in mice, maturity is monitored by surface expression of the CD28/CTLA4 ligands CD80 and CD86. Two distinct populations of mature rat hepatic DC have been identified: (1) $ED1^+ED2^-OX6^+$ and (2) $ED1^-ED2^-OX6^+$. In humans, DC are commonly MHC II^+ and deficient in CD28/CTLA4 ligands while in an immature state. Prickett and colleagues[7] found that human liver DC were also $CD45^+CD11a^+CD18^+$.

Thus it can be seen that there are similarities and disparities among DC populations. Common features to all three species include the lack of or low expression of MHC II and CD28/CTLA4 ligands on immature DC that are increased on maturation. CD11c and OX62 are generally considered the definitive markers for mouse and rat, respectively.

ENUMERATION OF HEPATIC DC

The normal murine liver, one of the larger visceral organs, has a relatively high total interstitial DC content, about 2–5-fold greater than that of other parenchymal organs, such as the kidney or heart.[38] However, when the density of MHC II^+ DC between these organs is compared, the liver ranks as the lowest.[38]

Specific DC populations, such as myeloid and lymphoid related subsets, studied in other tissues[24 39 40] (table 1), can be found in normal mouse livers. Previous studies have shown that these subpopulations constitute a low percentage of the total tissue specific DC population. The relative proportions of these two subsets in the liver are similar to those seen in other tissues.[12 24 39 40] Each population constitutes 1% of the total normal liver NPC population.[12]

Liver DC can be isolated from NPC by collagenase digestion followed by metrizamide density centrifugation.[12 15 41] Although the total number of DC in the liver is greater than that of other parenchymal organs, there are still few cells to work with in comparison with lymphoid tissue. This paucity of cells

Table 1 Phenotype of liver dendritic cells

Species	Maturation status	Markers	Comments (ref)
Mouse	Immature	$CD11c^+CD40^{lo}CD80^{lo}CD86^{lo}$MHC II^{lo}	2 subsets:[1 14 41 101]
		(1) $CD8\alpha^-CD11b^+$	(1) Myeloid related[12 102]
		(2) $CD8\alpha^+CD11b^-$	(2) Lymphoid related
		$B220^-CD11c^+CD205^-F4/80^-$	Yoneyama[65]
		$CD205^+OX2^+$	Gorczynski,[37] Drakes,[43] Gorczynski [103]
		$CD11b^+CD24^+CD44^+CD45^+CD11c^{lo}CD16/3\ 2^{lo}\ CD40^{lo}\ CD80^{lo}$ $CD86^{lo}CD205^{lo}F4/80^{lo}$	Generated from liver progenitor cells with GM-CSF; called liver derived DC progenitors.[32 33]
	Mature	$CD11c^+$MHC $II^{hi}CD86^{hi}$	
		$CD11c^+CD54^+CD205^+$MHC II^+CD11b^{mod} $CD86^{mod}CD11a/CD18^{mod}$ $B220^-CD3\epsilon^-Gr1^-$	Yoneyama[65]
	Other	$CD205^{hi}$ $B220^+CD11c^-$ $CD19^-$	Generated from liver progenitor cells with IL-3 and CD40L. Ig gene rearrangement occurs but no surface expression. Activate then subsequently induce apoptosis of T cells.[34]
Rat	Immature	MHC II^+ANAE$^-$FcR^-	ANAE=α-naphtylacetate esterase=a non-specific esterase; FcR=Fc receptor[53]
	Mature	MHC I^{hi}MHC $II^{hi}CD54^+OX62^+$	Brenan,[36] Matsuno,[56] Saiki[92]
		(1) $ED1^+ED2^-OX6^+$, (2) $ED1^-ED2^-OX6^+$	2 subsets of $OX62^+$ cells[104]
		MHC $II^+CD54^+OX62^+CD90^+$CTLA-4 Counter-receptor$^+$	Chen-Woan[35]
		$CD4^+$	Variable expression is common in MHC II^+ DC and peripheral tissues of rat[51 105]
Human	Immature	$CD11a^+$ $CD45^+$MHC II^+	Prickett[7]
		$CD83^{lo}CD86^{lo}$MHC II^{lo}	Ninomiya [84]
	Mature	$CD200^+$	Goddard[106]
		$CD83^+CD86^+$	Goddard[107]

DC, dendritic cell; GM-CSF, granulocyte macrophage-colony stimulating factor; IL, interleukin; MHC, major histocompatibility complex.

is especially evident if a specific DC subset is sought. Administration of recombinant human fms-like tyrosine kinase 3 ligand (Flt3-ligand, Flt3L), an endogenous haematopoietic growth factor, markedly increases the total number of hepatic DC.[12] Furthermore, the yield can be further increased by overnight culture of the isolated DC progenitors with GM-CSF. Under such culture conditions, the percentage of both $CD8\alpha^-$ and $CD8\alpha^+$ DC can be increased to 10–15% of the total NPC population.[12]

The phenotype of the DC obtained from Flt3L mobilised mice resembles that of DC isolated from normal liver and in situ.[12 15 33 41–43] Drakes and colleagues[43] showed that administration of Flt3L did not change the phenotype of freshly isolated hepatic DC, as defined earlier. These Flt3L treated DC, on culture with GM-CSF and IL-4 or exposure to a maturation inducing stimulus, such as extracellular matrix (ECM) protein, increased their surface costimulatory molecule expression and T cell allostimulatory activity.[33 43–45]

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The leucocyte content of the liver and its DC constituency in particular, appear to play an important role in transplant outcome. Thus when donor hepatic leucocytes are either drastically reduced[46–48] or greatly augmented,[49 50] a switch from tolerance to rejection occurs in murine liver transplantation. In the case of donor leucocyte depletion, transplant tolerance can be restored by replacement of donor leucocytes.[47] Thus a balance appears to exist between the number of donor hepatic DC and liver tolerogenicity.

APC FUNCTIONS OF HEPATIC DC

Phagocytosis

Early studies showed that intravenous administration of colloidal carbon[8 51 52] or antibody coated human red blood cells[53] did not result in phagocytosis by DC. It was speculated that liver DC, unlike KC and LSEC,[2 54 55] did not phagocytose these particles in vivo. However, more recently, elegant studies in the rat by Matsuno and colleagues[56 57] have shown that carbon laden DC localise in the coeliac nodes within two hours of intravenous administration of carbon particles. Furthermore, it was determined that immature DC were the major population of particle laden cells that entered the hepatic lymph. It was suggested that these phagocytic DC were recruited from the systemic circulation and were not part of the resident DC population. Interestingly, Iyoda and colleagues[58] have reported that in mice, only the liver resident $CD8\alpha^+$ DC subset exhibits phagocytic properties in situ.

T cell stimulation

Murine liver DC progenitors cultured overnight with or without GM-CSF stimulate naïve allogeneic T cells.[14 15 49] Abe and colleagues[13] observed that the allostimulatory activity of immature liver derived DC for memory T cells was not affected by administration of proinflammatory cytokines such as tumour necrosis factor α (TNF-α) or IFN-γ. However, addition of Ag (that is, viral antigen; keyhole limpet haemocyanin) to immature hepatic DC induced upregulation of MHC II, costimulatory molecules, and T cell allostimulatory activity. Khanna and colleagues[14] found that although cultured immature mouse liver derived DC were weak stimulators of allogeneic naïve T cells in vitro, their in vivo administration to allogeneic recipients resulted in selectively increased IL-10 production within secondary lymphoid tissue. By contrast, mature BM derived DC elicited increased IFN-γ but not IL-10 production. Immature hepatic DC therefore resemble freshly isolated immature respiratory tract DC that poorly stimulate allogeneic T cells and selectively induce Th2 responses.[59] These features of liver derived DC are consistent with hepatic "tolerogenicity" and may play a role in immune response deviation following liver transplantation.

There is as yet little documented information on the T cell stimulatory ability of purified freshly isolated human liver DC. Based on their immature phenotype in situ[60] (including lack of CD86) and the known properties of circulating peripheral blood DC with an immature phenotype,[61] it is likely however that these cells are weak allostimulators.

DC isolated from lymph

Matsuno and colleagues[56] have surveyed and analysed rat hepatic DC after they have exited the liver and entered the lymphatic circulation. By selective lymphadenectomy, it is possible to directly anastomose peripheral lymphatics to the thoracic duct, allowing draining cells to circumvent lymphoid tissues.[62] Thus non-lymphoid cells in peripheral lymph can be collected from the thoracic duct. Removal of coeliac nodes allowed enrichment of the lymphatics with hepatic DC, leading Matsuno and colleagues[62] to speculate that the liver is perhaps the greatest source of lymph from the gastrointestinal tract. The particle laden DC that entered the lymph were found to be non-phagocytic, even though they appeared immature cytologically. Furthermore, they were found to be strong T cell allostimulators. It has been suggested that these DC are in the early stages of maturation. Little is known of the activation, maturation, and migration of hepatic DC subsequent to Ag uptake.

Portal tract associated lymphoid tissue (PALT)

Portal lymphoid follicles were described in chronic active hepatitis C as early as 1992.[63 64] These areas of B and T cell interactions exhibit many histological features classic to lymphoid follicles. More recently, Yoneyama and colleagues[65] have identified DC-T cell interactions within these specialised areas of the liver. On infection with *Propionibacterium acnes*, granulomas form within the liver. DC are mobilised to these sites and can be found to (1) traffic to the hepatic LN; (2) remain in the developing sinusoidal granuloma; or (3) associate with immunoresponsive cells (B and T cells, DC) in a distinct area near the portal triad, termed the PALT by Yoneyama *et al*.

> "Portal inflammation and PALT development have been identified in primary sclerosing cholangitis"

This lymphoid tissue-like area comprises B cell follicles with follicular DC (not BM derived DC but DC specialised for the presentation of Ag captured in immune complexes) interspersed throughout the follicles. $CD4^+$ T cells were found to localise between B cell follicles, but not within these structures, unlike the broad distribution seen within sinusoidal granulomas. Surrounding the B and T cell areas were macrophages. In patients with hepatitis C virus infection, plasma cells and B cells are also found in association with DC within hepatic portal areas, as in lymphoid tissue.[66] Similarly, portal inflammation and PALT development have been identified in primary sclerosing cholangitis (PSC).[67 68] CCL21 (secondary lymphoid chemokine), a lymph node associated chemokine, is upregulated on $CD34^+$ vascular endothelium of PALT. Expression of CCL21 recruits $CCR7^+$ cells that commonly include DC and naïve T cells.[69 70] These findings suggest that there may be important immune cell interactions occurring within PALT, perhaps circumventing the need for DC migration to lymphoid tissue.

Liver derived DC progenitors

In order to generate DC from normal liver, Lu and colleagues[33] applied a procedure introduced for the propagation of DC from murine blood or BM. Inaba and colleagues[71 72] first showed that culture of normal mouse BM cells with GM-CSF resulted in the propagation of DC. Similarly, culture of liver NPC yielded a population of replicating DC progenitors.[33] These immature DC exhibited classic veiled morphology, high surface expression of CD45, CD11b, CD24, and CD44, moderate to low expression of CD11c, CD16/32, CD54, CD205, and F4/80, and low expression of the costimulatory molecules CD40, CD80, and CD86. Furthermore, these cells were resistant to typical DC maturation inducing stimuli, such as the proinflammatory cytokines IFN-γ and TNF-α. Extended culture failed to upregulate MHC II or costimulatory molecules. Instead, these cells matured in response to ECM proteins, such as collagen type 1[13 33 44 45] (with which DC are associated spatially in normal liver), losing their phagocytic ability and gaining the ability to stimulate naïve allogeneic T cells.

A novel population of mouse liver derived DC-like cells has been propagated in response to IL-3 and CD40L.[34] These cells have a phenotype and function distinct from typical immature or mature myeloid or lymphoid related mouse DC. Ig rearrangement occurs within these cells without surface expression of Ig molecules. Furthermore, they have a distinct pattern of surface markers and maintain a DC-like morphology. These $CD205^{bright}CD11c^-B220^+CD19^-$ cells activate T cells and promote their apoptosis. Lu and colleagues[34] also showed that a T regulatory type 1 cytokine expression pattern was induced by these DC.

Chemotaxis

Migration of DC to and from peripheral tissue depends on the production of chemokines (CC and CXC) and expression of specific chemokine receptors (CCR and CXCR). Because leucocyte migration is a key event in infection and inflammation, chemokine biology is rapidly becoming an important area of study in relation to elucidation of DC function. Most chemokine receptors are promiscuous and can ligate a variety of different chemokines.[73–75]

> "Because leucocyte migration is a key event in infection and inflammation, chemokine biology is rapidly becoming an important area of study in relation to elucidation of DC function"

In the case of hepatic DC, few studies have been conducted regarding specific chemokine and receptor expression. Drakes and colleagues[76] showed that immature and mature liver derived DC exhibited similar chemokines and receptors, although with differing levels of expression. Expression was similar to that detected on BM derived DC. As determined by the RNase protection assay, the chemokine most strongly expressed by both immature and mature liver derived DC was CCL5 (RANTES, regulated upon activation, normal T cell expressed and secreted). However, CCL3 (MIP-1α, macrophage inflammatory protein 1α), CXCL1 (MIP-2), and CCL2 (MCP-1, monocyte chemoattractant protein 1) were also expressed by these liver derived DC. Receptors CCR1 and CCR2 were expressed at comparable levels on these liver DC. CCL5 and CCL3 are among the various chemokines that bind CCR1 while CCL2 binds CCR2. CCL3 expression was greatly enhanced on liver DC maturation and stimulation by bacterial lipopolysaccharide or naïve allogeneic T cells also induced chemotaxis of mature liver derived DC.

Shields *et al* found that CCR5, for which CCL3 is a ligand, is important in T cell recruitment in both hepatitis C virus (HCV) infected and normal livers.[77] Goddard *et al* similarly observed the importance of CCR5 in T lymphocyte recruitment during the inflammatory response in human liver transplantation.[78] The presence of this receptor on T cells coupled with the production of CCL3 by resident liver cells implies the existence of DC-T cell interactions within the liver under normal and inflammatory conditions. Further studies are needed to assess the role of chemokines and their receptors in the regulation of hepatic DC migration and function.

HEPATIC DC AND LIVER DISEASE

Viral hepatitis

Both hepatitis B and C viruses (HBV and HCV, respectively) are major health concerns as these are not only infectious diseases with distinct pathogeneses but are also major prognostic factors for hepatocellular carcinoma (HCC). Although some studies

have investigated the role of resident liver DC in defence against these viruses, there is still much to understand.

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A general agreement in the literature is the existence of dysfunctional DC in both HBV and HCV infection. HBV transgenic mice that express HBV Ag are used as a model for chronic HBV carriers. These mice show low immune efficiency, as defined by decreased overall specific antibody responses and lowered DC allostimulatory capabilities.[79 80] In one study, it was found that defective splenic DC had low costimulatory molecule expression and low IL-12 production.[80]

Peripheral blood derived DC from chronic HCV patients show impaired maturation.[81 82] They fail to respond to TNF-α (that typically induces DC maturation) and are poor T cell allostimulators. These cells also show decreased production of bioactive IL-12 p70.[81] Less aggressive HCV infection is associated with inflammation, confined mainly to portal areas where hepatic DC generally reside. In contrast with reports of increased immature blood DC in chronic HCV patients, electron microscopy and surface marker expression have identified the portal infiltrate DC as phenotypically mature.[66] These DC are also associated with the formation of new lymphatic capillaries within chronic hepatitis C livers.[66] Thus Galle *et al* speculate a critical role for DC in mediating the HCV disease state based on increased lymphatic drainage and the association of DC with these sites.

One of the chemokines present in portal areas during HCV infection is CCL3.[77] This chemokine is produced by T cells, macrophages (KC), and fibroblasts, and attracts DC as well as T cells. Other chemokines present in the portal area at sites of piecemeal necrosis in patients with chronic hepatitis C include CCL5 and DC-CK1, which have been correlated with an active immune response against HCV.[83] In fact, DC-CK1 is found in the PALT. It is possible that the production of these cytokines aides DC-T cell interactions.

Hepatocellular carcinoma (HCC)

A prerequisite for effective immune responses against tumours is the need for cells that recognise, process, and present tumour Ag. DC are considered promising biological therapy agents for cancer treatment. In patients with HCC, there is evidence that immature DC with maturation defects are the predominant type of peripheral blood DC.[84] Circulating DC show reduced expression of HLA-DR and IL-12 and reduced endocytotic and allostimulatory capacity.[84] Additionally, these DC remain immature in the presence of high levels of inflammatory cytokines that normally induce DC maturation.[84]

"DC are considered promising biological therapy agents for cancer treatment"

By contrast, it has been reported that activated $CD83^+$ DC are increased in the peripheral blood of HCC patients compared with normal patients and patients with liver cirrhosis.[85] However, total DC are reduced in the livers of HCC subjects and not localised to cancer nodules.[85] Importantly, it had been shown that administration of Flt3L can drastically reduce the number of hepatic metastases in experimental animals.[86] Tumour borders exhibited increased infiltration with both DC and T cells as well as increased apoptotic bodies. Thus DC may have an important role in surveillance and clearance of tumour cells in liver cancer.

Granulomatous liver disease

Recent studies have revealed DC recruitment to hepatic sites of experimental granulomas. Yoneyama and colleagues[65] observed $CD11c^+F4/80^-B220^-$ DC in *P acnes* induced granulomas in the perisinusoidal space. These DC later interacted with T cells in the PALT.

Autoimmune diseases

Patients with primary biliary cirrhosis (PBC) have dysfunctional DC with increased production of nitric oxide and lowered allostimulatory capability.[61] The number of DC present in portal tracts is greater in PBC patients compared with HCV patients.[60] Kaji *et al* also found that these CD86 positive DC appeared to be more relevant in the earlier stages of PBC as they disappeared from the liver at later stages.

TRANSPLANTATION

The immunobiology of liver transplantation has long been a field of intense study as it may provide valuable insight into the mechanisms underlying transplant tolerance. Liver transplant patients are known to achieve graft acceptance without continued immunosuppressive drug therapy. Moreover, graft failure due to chronic rejection is rare compared with other types of organ transplantation. Furthermore, liver transplantation can protect other organ grafts from the same donor transplanted in conjunction with the liver. Pigs, mice, and some rat strain combinations will accept liver allografts across MHC barriers without immunosuppressive therapy. This acceptance may be lost by removal of donor leucocytes prior to liver transplant[46 47] while replacement of donor leucocytes abrogates rejection.[47] These findings suggest that donor leucocytes, that include DC, have the capacity to modulate host anti-donor immune reactivity.

"Donor leucocytes, that include DC, have the capacity to modulate host anti-donor immune reactivity"

There are several concepts that attempt to explain the comparative privilege of liver allografts. Starzl *et al* have proposed the theory of microchimerism (two way silencing of immune reactivity, linked to deletion of alloreactive T cells) to explain promotion of tolerance induction.[22 87 88] Microchimerism is the persistence of donor haematopoietic cells within both lymphoid and non-lymphoid tissues of the host. Significantly, donor derived DC can be propagated from the blood or BM of liver transplant recipients.[89 90] In mice, this is achieved in liver recipients that accept these grafts without immunosuppression and that develop donor specific tolerance, but not in mice that acutely reject heart grafts from the same donor strain.[89]

The liver is a haematopoietic organ and thus compared with other transplanted organs may have an advantage in being a continuous source of donor haematopoietic stem/progenitor cells. Many circulating haematopoietic cells also take up residence in the liver. In addition, three hepatic stem cell candidates have been described to date: fetal progenitor bipotential hepatic stem cells, adult hepatocytes, and oval cells—a type of non-parenchymal pluripotent hepatic stem cell.[91] The existence of these liver stem/progenitor cells suggests that a hepatic progenitor cell exists for the production of liver specific DC in situ. Importantly, donor interstitial DC appear to self replicate in rat liver graft recipients.[92] These donor derived immature DC may promote donor specific tolerance induction.

It has been argued on the other hand that comparatively large numbers of donor leucocytes present in liver allografts cause overstimulation or "abnormal" early activation of recipient T cells that leads to their exhaustive proliferation and deletional tolerance.[48]

"Conceivably, donor DC may play a role in inducing apoptosis in host T cells via death ligand receptor pathways"

There are many potentially important mechanistic roles for the hepatic DC in determining the outcome of transplantation. Alloreactive host T cell apoptosis in experimental liver transplantation is associated with tolerance whereas less apoptosis is seen with rejection.[93–95] Conceivably, donor DC may play a role in inducing apoptosis in host T cells via death ligand receptor pathways.[96] It has been shown that there may be such a role for tissue resident migratory DC in immune privileged sites, such as the anterior chamber of the eye[97] or testis.[98] Neutralisation of IL-12 produced by liver resident DC and other APC in murine livers transplanted from Flt3L treated donors (that are rejected acutely) restores long term allograft survival and enhances alloreactive T cell apoptosis.[99] This suggests that suppression/inhibition of donor DC function promotes tolerance induction. The immature state of normal liver derived DC, associated with failure to provide adequate costimulation, may be important in inherent liver tolerogenicity. The immature state/absence of costimulation can also be achieved using immunomodulatory agents, such as IL-10[19] or CTLA4-Ig.[23] Administration of liver DC progenitors prior to transplantation has been shown to increase allograft survival, although not to induce tolerance.[34 100] It remains to be determined whether in a clinically relevant large animal (primate) model, coadministration of immature donor DC with appropriate pharmacological agents or biological immunosuppressants that inhibit their maturation and those of recipient DC would promote the induction of organ transplant tolerance.

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