

LEADING ARTICLE

Liver tolerance mediated by antigen presenting cells: fact or fiction?

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Possible mechanisms by which liver antigen presenting cells (APC) may facilitate tolerance induction are discussed. Tolerance may be facilitated by a distinctive combination of factors, linked to the unique anatomical and microenvironmental features of the liver.

It is generally accepted that following organ transplantation, rejection mediated by alloreactive T cells will occur if donor and recipient are mismatched for so-called transplantation antigens (Ag), especially those encoded by the major histocompatibility complex (MHC). These rules do not always apply to liver transplantation as hepatic allografts may be accepted "spontaneously" in inbred mice, rats, and outbred pigs without the need for, or following withdrawal of, immunosuppression, even across full MHC barriers. Moreover, spontaneous acceptance of experimental liver grafts is accompanied by donor specific tolerance of other transplanted organs, such as the kidney or heart. The concept of liver "tolerogenicity" in humans is supported by evidence that hepatic allografts have a protective effect on the survival of other organ transplants performed at the same time¹ and that, unlike with other organs, MHC matching is not a prerequisite for successful transplant outcome. Furthermore, safe and lasting withdrawal of a proportion of stable liver transplant recipients from all anti-rejection therapy has been achieved under physician directed protocols.^{2,3}

The tolerogenic properties of the liver may have evolved in response to the need for immunological unresponsiveness to orally acquired Ag presented by Ag presenting cells (APC) and may explain the persistence of some viral infections within the liver as well as the survival advantage of liver allografts. Mechanisms proposed to account for the tolerogenic properties of the liver have not yet been validated convincingly, and indeed its inherent tolerogenicity may be due to a combination of several biological properties that distinguish the liver from other parenchymal organs. Thus in addition to its unique structure and vascular architecture that facilitates interaction between liver parenchymal and non-parenchymal cells and circulating immune cells, the liver also exhibits extensive regenerative and haematopoietic potential. An association between the long term persistence of donor derived haematopoietic cells and induction of tolerance has been demonstrated in liver graft recipients.⁴ Moreover, it has been speculated that donor derived dendritic cells (DC) generated from

precursors present in the liver may play a key role in the subversion of T cell mediated immunity and the long term survival of liver allografts.⁵

The diverse constituency of hepatic professional and non-professional APC, that include liver sinusoidal endothelial cells (LSEC), liver macrophages (Kupffer cells; KC), hepatocytes, and DC, may play key roles in regulating immune responses and facilitating tolerance induction. The unique architecture of the liver allows T cells to interact with resident liver APC, making these latter cells, particularly the uniquely well equipped DC, ideal candidates for directing immune responses towards immunity or tolerance. We speculate that within the liver microenvironment, production of anti-inflammatory cytokines and other molecules including growth factors, such as interleukin (IL)-10, transforming growth factor (TGF)- β , prostaglandin (PG)E₂, and granulocyte macrophage-colony stimulating factor results in the modulation of APC differentiation, trafficking, and function in both health and disease. Much has recently been discussed about the potential of DC to regulate immune responses and to promote tolerance induction. The aforementioned soluble factors may each play a role in "fashioning" intrahepatic tolerogenic DC and other APC.

Due to their capacity to express death ligands, DC and other APC may also contribute to the extensive apoptosis of systemically activated T cells within the liver, a phenomenon that may underlie inhibition of anti-donor responses following hepatic transplantation. The number of T cells that undergo apoptosis within the early inflammatory infiltrate of murine liver allografts is increased significantly compared with grafts that are rejected acutely.⁶ Both activation induced cell death and passive cell death may be involved in T cell apoptosis that predisposes to tolerance induction. Although CD8⁺ T cell apoptosis has been shown to be Fas-FasL (CD95-CD95L) pathway independent, alternative death receptor pathways have not been excluded. Apoptosing T cells release TGF- β and IL-10,⁷ further contributing to the anti-inflammatory microenvironment of the liver. Immune reactivity may also be

Abbreviations: Ag, antigen; MHC, major histocompatibility complex; APC, antigen presenting cells; DC, dendritic cells; LSEC, liver sinusoidal endothelial cells; KC, Kupffer cells; IL, interleukin; TGF, transforming growth factor; PG, prostaglandin; NK, natural killer; TNF, tumour necrosis factor; Th, T helper; Treg, regulatory T cells; IFN, interferon; CTLA4lg, cytotoxic T lymphocyte Ag 4 immunoglobulin.

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affected by interactions between DC and natural killer (NK) cells, NK T cells, and $\gamma\delta$ T cells that are found in unusually high proportions within the liver. Hereon, we will highlight and discuss possible mechanisms by which liver APC may facilitate tolerance induction.

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As the liver surveys a massive blood supply, mainly first pass blood from the gut, it is, compared with other organs, constantly exposed to a vast quantity and diversity of Ag. Among the different cell populations thought to be involved in hepatic tolerance, LSEC are also important because they are highly efficient sessile APC⁸ with immunoregulatory functions. Unlike vascular endothelial cells from other organs, such as skin, gut, or lung, which cannot function as APC for CD4⁺ and CD8⁺ T cells unless pre-exposed to proinflammatory cytokines, LSEC can cross present exogenous Ag⁹ and induce Ag specific tolerance.¹⁰ The consequence of cross presentation of Ag by LSEC in vivo is induction of systemic immune tolerance through clonal deletion as well as induction of T cell anergy.¹⁰

LSEC express surface molecules necessary for efficient Ag uptake by receptor mediated endocytosis, interaction with leucocytes, and efficient Ag presentation to T cells. In addition, LSEC can express apoptosis inducing molecules (FasL, tumour necrosis factor (TNF) receptor apoptosis inducing ligand (TRAIL), and membrane TNF- α) that may contribute to intra-hepatic T cell death.¹¹ In vitro, mouse LSEC can prime T cells efficiently but the tendency is towards tolerance induction. Thus murine CD8⁺ T cells activated by LSEC are functionally inert, lack cytotoxic effector function, exhibit poor cytokine release, and eventually apoptose¹⁰ whereas CD4⁺ T cells differentiate into IL-4 secreting T helper (Th)2 cells or IL-10 secreting regulatory T cells (Treg).¹² Furthermore, in the presence of IL-10 or PGE₂, murine LSEC downregulate MHC class II and costimulatory molecule expression and decrease mannose receptor mediated Ag uptake, which results in diminished T cell activation.¹³ Human LSEC, on the other hand, do not normally express MHC class II or costimulatory molecules and it is reasonable to assume that T cells are not activated by these APC under non-inflammatory conditions.¹⁴ Not only are IL-10 and PGE₂ important in promoting the tolerogenic properties of LSEC, but they are also expressed constitutively by these cells and upregulated upon stress.^{13, 15} Thus modulation of T cell activation by LSEC is a likely reflection of the influence of IL-10 or PGE₂ on the Ag presenting capacity of these cells.

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KC, the resident liver macrophages, phagocytose microorganisms efficiently and initiate T cell responses. In addition, like DC, they may also play a role in the induction of tolerance via mechanisms such as cross presentation of Ag derived from ingested apoptotic cells¹⁶ to either resident DC^{17, 18} or DC traversing the liver. Classical macrophage activation is induced by interferon (IFN)- γ and by microbial triggers such as bacterial lipopolysaccharide, and results in secretion of proinflammatory cytokines (TNE, IL-1, IL-6, IL-12), nitric oxide, and upregulation of MHC class II molecules.¹⁹ However, KC may also be deactivated under the influence of factors within the liver microenvironment. Indeed, it has been shown for other types of macrophages, such as those derived from human blood mononuclear cells,²⁰ that anti-inflammatory cytokines

such as IL-10 and TGF- β inhibit their ability to secrete proinflammatory factors (IL-12, IFN- γ), downregulate MHC class II expression, induce PGE₂ production, and promote paracrine and autocrine secretion of IL-10 and TGF- β .¹⁹ Furthermore, KC appear to play a crucial role in tolerance induction induced by portal venous administration of soluble alloAg.²¹

“DC are the best equipped and, when mature, the most potent APC”

DC are the best equipped and, when mature, the most potent APC. There is now considerable evidence for their phenotypic heterogeneity and functional diversity. Their activities are modulated by a range of stimuli, including pathogen derived products and inflammatory mediators. Depending on Ag dose, their maturation state, and environmental signals, DC can direct either Th1- or Th2-type immune responses. They are rendered tolerogenic by exposure to immunosuppressive cytokines (IL-10 and TGF- β) that inhibit production of the Th1 driving cytokine IL-12 and suppress costimulatory molecule expression. Blocking of costimulatory molecules on the surface of DC^{22, 23} (for example, by cytotoxic T lymphocyte Ag 4 immunoglobulin (CTLA4Ig)) inhibits their T cell stimulatory ability and promotes death of alloreactive T cells.²⁴ Induction of Treg cells by immature DC is also a possible mechanism that may contribute to induction of tolerance. It has been shown that the presence of Treg cells within allografts is important in transplant tolerance.²⁵ Activation of Treg cells is believed to downregulate MHC class II and costimulatory molecules on DC, maintaining them in an immature state.²⁶ In addition, repetitive interaction of allogeneic T cells with immature DC leads to expression of CTLA4, IL-10 production, and induction of a Treg phenotype.²⁷ In humans, injection of immature autologous DC pulsed with antigenic peptide induces Ag specific tolerance in CD8⁺ T cells.²⁸

“An important factor contributing to a role of liver APC in the promotion of tolerance is the unique hepatic microenvironment that is rich in IL-10 and TGF- β ”

We contend that an important factor contributing to a role of liver APC in the promotion of tolerance is the unique hepatic microenvironment that is rich in IL-10 and TGF- β (synthesised by hepatocytes, LSEC, and other liver cell types, including T cells). In particular, this environment may sustain or promote an immature/tolerogenic phenotype in resident DC. Indeed, it has been shown that freshly isolated hepatic DC are poor stimulators of naïve allogeneic T cells and, when administered in vivo, promote IL-10 production selectively in secondary lymphoid tissue.²⁹ We postulate that potentially tolerogenic APC of donor or recipient origin (the latter trafficking into the graft) may play a key role in the acceptance of liver allografts by inducing Treg cells and/or T cell apoptosis. Indeed, the unique microenvironment of the liver may provide an ideal site for the immature (tolerogenic) DC-T cell inhibitory feedback loop described recently by Min and colleagues.³⁰

In addition to their maturation status, the nature of the DC subsets present in the liver may contribute to its potential tolerogenicity. Mouse liver contains several DC subsets, including myeloid (CD8 α), lymphoid related (CD8 α ⁺), and the recently described plasmacytoid (B220⁺) DC.^{31, 32} CD8 α ⁺ DC have been implicated in the induction of central and peripheral tolerance. They express FasL, induce apoptosis in alloactivated CD4⁺ T cells, and inhibit IL-2 production by CD8⁺ T cells.^{33, 34} In addition, they prolong heart allograft survival independent of their maturation status.³⁵ Another subset of DC-like cells that are generated by the culture of hepatic non-parenchymal cells

with IL-3 and CD40L may be involved in promoting T cell apoptosis. These DEC205⁺CD11c⁺B220⁺CD19⁻ cells have been shown to first activate and then to induce T cell apoptosis.³⁶

A possible role of plasmacytoid DC, identified recently in murine liver (Lau AH *et al*, unpublished data), in immune regulation has not yet been addressed experimentally. Previous data suggest that these DC may have a regulatory function in their immature state in which they can promote CD4⁺ T cell anergy.³⁷ Furthermore, human plasmacytoid DC appear to inhibit T cell proliferation by secretion of the tryptophan catabolising enzyme indoleamine 2,3-dioxygenase³⁸ and CD40 ligand activated human plasmacytoid DC generate IL-10 producing CD8 Treg cells.³⁹ These data suggest a possible role for liver plasmacytoid DC in liver tolerance.³⁹

A further consideration is the influence of the liver microenvironment on trafficking of APC. BM derived DC cultured in the presence of IL-10 upregulate expression of message for the chemokine receptor CCR5 while downregulating CCR7 mRNA. This corresponds with impaired homing ability of the DC to secondary lymphoid tissue.^{40,41} These data suggest that, in the presence of IL-10, liver DC could have reduced or defective ability to migrate to secondary lymphoid tissue. Such impaired migration could undermine induction of anti-donor responsiveness due to a reduced ability of the DC to reach T cell areas of the draining lymphoid tissue.

Following liver transplantation, massive Ag release occurs as a result of physical and ischaemic tissue injury, hepatocyte production of donor MHC class I Ag,⁴² and the migration of donor haematopoietic cells into the host. This extensive Ag release likely results in the binding of soluble donor Ag by host T cells—a fact that may block their recognition of target cells, suppress anti-donor reactivity, and promote tolerance induction.

“Tolerance may be facilitated by a distinctive combination of factors, linked to the unique anatomical features of the liver”

Mechanisms by which the liver may foster immunological tolerance under normal steady state conditions and in the context of liver transplantation can be inferred from its unique constituency of APC and their presence in a cytokine microenvironment conducive to their tolerogenic function and potential to subvert T cell responses. Tolerance may be facilitated by a distinctive combination of factors, linked to the unique anatomical features of the liver. Conventional and experimental immunosuppressive agents that inhibit both APC and T cell activation and function are likely to contribute significantly to hepatic tolerogenicity.

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