Review

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Liver antigen-presenting cells

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The liver is an organ in which several major pathogens evade immune clearance and achieve chronicity. How do they do it? Recent research has documented multiple mechanisms by which immune responses in the liver are biased towards tolerance. In this review, the induction of local, intrahepatic tolerance is explored from the perspective of antigen presentation. Experiments support the role not only of liver dendritic cell subsets but also of diverse subsets of unconventional antigen-presenting cells in inducing immune suppression. The literature on this topic is controversial and sometimes contradictory, making it difficult to formulate a unified model of antigen handling and T cell priming in the liver. Here I offer a critical review of the state of the art in understanding antigen presentation in the liver.

Introduction

The liver is the site of several infections of major importance, against which the immune system normally delivers either an ineffective or a pathogenic response. In the case of Hepatitis B and Hepatitis C, immune responses occur but they are frequently ineffective. With the lack of virus elimination, chronic immune responses cause cumulative tissue damage and eventual fibrosis, leading to disruption of the liver's hemodynamics, and the loss of liver function. In contrast, malaria parasites migrate through the liver and undergo an essential part of their maturation there, yet there is no evidence of an endogenous immune response. While much of the understanding of human liver immunology is based on the study of immune responses to viral hepatitis, the world's most prevalent serious infection, malaria, is also a liver pathogen. Most species of murine, and all human malaria parasites undergo an obligatory developmental stage in the liver. The sporozoite, introduced by the bite of an infected mosquito, interacts sequentially with the liver sinusoidal endothelial cells [66] and Kupffer cells [65]. The Kupffer cells appear to be an essential "gateway" through which sporozoites penetrate the endothelial barrier and enter the hepatocytes [4]. Once in the hepatocytes, the parasites develop rapidly over several days, after which the host cell dies and merozoites are released, which in turn parasitize red blood cells. The liver stage is an attractive vaccine target, and genetically modified murine malaria parasites create sterilizing immunity that appears to intercept the infection at the liver stage [57]. The mechanism of action of the vaccine is not understood, but it appears to depend on Interferon (IFN)-γ, and on CD8+ T cells [30].

In many mammalian species, the transplantation of the liver across a Major Histocompatibility Complex (MHC) difference does not result in rejection [13,15]. This stands in contrast to the consequences of transplanting kidneys, skin, pancreas or other organs, where rejection is the usual outcome. In addition, the transplanted liver is able to confer tolerance on another solid organ transplant from the same donor, arguing that the liver can induce systemic tolerance [14]. This effect is not fully understood, but it has been attributed to: the effects of liver-derived APC dispersed throughout the host, also known as microchimerism [67]; the effects of Kupffer cells or liver sinusoidal endothelial cells (LSEC) as antigen-presenting cells (APC), promoting tolerance [38,70]; the distinctive properties of liver-resident dendritic cells (DC) [53]; and the induction of allospecific regulatory T cells of the CD4+, CD25+, Forkhead transcription factor-P3 (FoxP3)+ type [46]. Despite the well-documented liver allograft tolerance in many animal models, human liver transplants are undertaken with the use of immunosuppressive drugs. In the context of a pathogen that re-infects the liver allograft, such as Hepatitis C Virus, this situation leads to rapid progression of the infection [54].

Clearly, the liver is a tissue in which immune responses are often suboptimal. How does this arise? Is the liver intrinsically predisposed towards immune tolerance, or is it simply that these pathogens are unusually adept at subverting host defense? The field of liver immunology addresses these issues by asking how far the liver has unique immunological properties. One key issue is that of antigen presentation; the liver contains DC and resident mononuclear phagocytes, but there is evidence that other hepatic cell types act as APC. In particular, there is extensive literature on the immunological properties of LSEC [38], while an influential recent paper argues that the liver's distinctive vascular pericytes, termed stellate cells or Ito cells, are capable of antigen

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Abbreviations: IFN, Interferon; MHC, Major Histocompatibility Complex; LSEC, liver sinusoidal endothelial cells; APC, antigen-presenting cells; DC, dendritic cells; FoxP3, Forkhead transcription factor-P3; LPS, lipopolysaccharide endotoxin; NK, natural killer; ICAM-1, Intercellular Cell Adhesion Molecule-1; VCAM-1, Vascular Cell Adhesion Molecule-1; CXCR6, C-X-C chemokine receptor-6; TLR, Toll-like receptor; mDC, myeloid DC; pDC, plasmacytoid DC; BDCA, blood dendritic cell antigen; B220, B cell isoform of CD45 of mass 220kd; LDL, low-density lipoproteins; HUVEC, human umbilical vein endothelial cells; Tie-2, endothelium-specific receptor tyrosine kinase type-2.

presentation [83]. Some evidence also suggests that hepatocytes, the metabolic engines of the liver, can under certain circumstances activate naïve T cells [9]. If this interpretation is correct, the analysis of immune responses to liver pathogens needs to take into account the possibility that unconventional APC play an important role, and may account for the failure of effective immunity. The purpose of this review is to critically evaluate the claims of each population of liver cells to be APC, and to ask how they may contribute both to effective and to maladaptive response to liver antigens.

Box 1. Central features of main liver APC

- Myeloid DC: appear to be biased towards tolerance induction
- Plasmacytoid DC: secrete type 1 IFN but are relatively poor APC
- Other DC: CD8-α expressing "lymphoid-like" DC, and NK-DC of uncertain lineage
- Liver sinusoidal endothelial cells: potent in cross-presentation of soluble and cellular antigens
- Hepatic stellate cells: Good APC for NK-T cells, but the data are contradictory for other T cells
- Cholangiocytes: express surface molecules like APC but do not activate T cells
- Hepatocytes: engage CD8+ T cells, cause abortive activation and early apoptosis

The hepatic vasculature and leukocyte trafficking

The liver is a major focus of metabolic activity, where the products of digestion are processed, plasma proteins synthesized, and dangerous foreign chemicals detoxified. To serve these functions, the liver receives its blood supply from two sources: around 20% of the blood is arterial, delivered via the hepatic artery which branches off from the celiac axis; while the other 80% originates in the intestine. This portal venous blood carries to the liver a mixture of antigens from food and bacterial products from the intestinal bacteria. In particular, the portal blood carries lipopolysaccharide endotoxin (LPS) at concentrations of up to 1 ng/ml [22,50]. Thus, in the liver, both antigen-specific lymphocyte receptors and pattern recognition receptors are exposed to their ligands.

The liver contains a diverse population of both adaptive and innate immune cells. T cells are abundant, with a bias towards CD8+ T cells, and activated T cells predominate [17,78]. Natural killer (NK) cells are abundant, and these cells similarly express activation markers [78]. NK-T cells are more frequent in the liver than in the blood in humans, and more frequent than in the lymphoid organs in mice; this holds true whether these cells are defined expansively as NK1.1+ T cells in the mouse, or CD56+ T cells in the human, or CD1d-reactive cells, or narrowly defined as T cells that bind tetramers of a glycolipid, α-galactosyl ceramide, associated with a CD1d molecule [6,59]. Lymphocytes with exactly these features can be eluted from the hepatic vasculature of a human liver lobe prior to transplant [78], suggesting that they are found in the lumen of the blood vessels, and immunohistology similarly reveals individual T cells through the normal

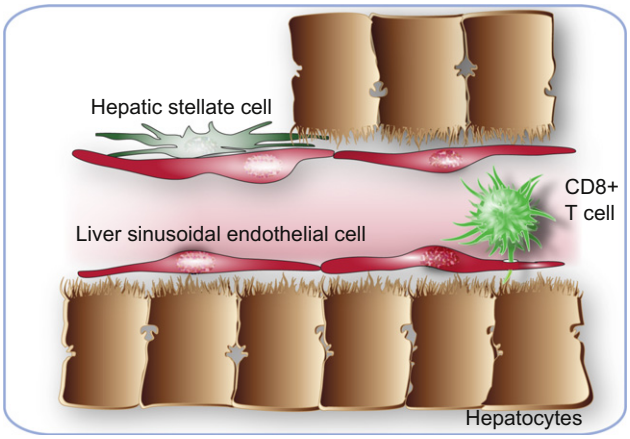

Fig. 1. Immunological players in the hepatic sinusoid. The liver sinusoidal endothelial cells are penetrated by holes (fenestrations), through which a CD8+ T cell can make direct contact with an underlying hepatocyte. Between the endothelial cells and the hepatocytes is the Space of Disse, in which reside hepatic stellate cells (Ito cells), a specialized pericyte with immunological properties. These cells respond to TLR ligands and synthesize chemokines, and they may also act as antigen-presenting cells, particularly for CD1d-restricted NK-T cells.

human liver parenchyma, as well as in portal tracts [75]. For most of these cell populations, it is not possible to say how far these cells are long-term hepatic residents, and how far they are preferentially slowed down in the liver during their recirculation by adhesion molecules on the hepatic endothelium. The liver has the capacity to preferentially sequester activated CD8+ T cells from the circulation [55], and this effect depends in part on Intercellular Cell Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1) expressed on the hepatic vasculature [31]. However, in the specific case of NK-T cells, *in vivo* microscopy was used to identify these cells in the living liver, exploiting their expression of CXCR6 and a knock-in strategy to render them fluorescent. These cells were observed patrolling the hepatic sinusoids, both with and against the direction of blood flow [24]. Activation of these cells causes them to stop patrolling, consistent with them having a defensive function [80]. These cells, at least, were not passively drifting through the liver, and are likely to be long-term residents.

Blood percolates through the liver in thin-walled vessels termed sinusoids, the endothelium of which is penetrated by small holes (fenestrations) grouped in clusters (sieve plates). The fenestrations are large enough to permit contact between lymphocytes in the blood space, and the underlying hepatocytes (Fig. 1). Electron micrographs show contact between T cell microvilli and their counterparts on the hepatocytes [82], though the physiological significance of such interaction is not clear. Certainly, these contacts are not sufficient to allow the formation of an immunological synapse, but in the living sinusoid they may act as initiators of more intimate contact. This progression has not yet been observed directly. The flow of blood is slow, due to the large cross-sectional area of the sinusoidal bed, and this is likely to facilitate interactions with both intra-sinusoidal and peri-sinusoidal cells. Some electron micrographs reveal gaps in the endothelial layer, but it is likely that in the living sinusoid, the liver's resident macrophage population, Kupffer cells, occupies these gaps. We know this partly because elimination of the Kupffer cells using toxic liposomes results in gaps in the endothelial barrier, through which malaria sporozoites gain easy access to hepatocytes, bypassing their usual route through Kupffer cells [4].