Control of immune responses by scavenger liver endothelial cells

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Summary

The liver appears to be an organ favoring the induction of immune tolerance rather than immunity. Among the hepatic cell populations possibly involved in regulation of immune responses, liver sinusoidal endothelial cells (LSEC) are well suited to fulfill this role. LSEC are resident cells lining the hepatic sinusoidal wall and therefore are in intimate contact with leukocytes passing through the liver. They are equipped with numerous scavenger receptors rendering antigen-uptake in these cells extremely efficient. Antigen processing and MHC-restricted presentation of exogenous antigens for CD4 as well as CD8 T cells occurs equally with high efficiency. Importantly, CD4 and CD8 T cells that engaged in cognate interaction with LSEC have a tolerant phenotype. Thus LSEC contribute an important immune function to the liver: control of the immune response against circulating soluble antigens.

Key words: immune response; liver sinusoidal endothelial cells; tolerance

Introduction

The “decision” whether to mount immunity or tolerance towards an antigen is an important issue for the immune system. While immunity needs to be generated against infectious pathogens, immune responses against innocuous or self-antigens are not desirable and need to be prevented. Deletion of auto-reactive T cells in the thymus is one mechanism assuring absence of auto-aggression. However, not all auto-reactive cells are eliminated in the thymus, thus requiring mechanisms of induction of immune tolerance in the periphery. Dendritic cells are the key-players in the induction of immunity. They perform their immuno-stimulatory function in lymphatic tissue, which provides an optimised anatomical platform for interaction between antigen-presenting dendritic cells and lymphocytes. Antigen-presenting cell populations involved in induction of immune tolerance appear to be more diverse. Moreover, different organs have different requirements with regard to control of immune responses which may be reflected by different, perhaps even organ-resident cell types that influence the immune response locally.

The liver seems to favour the induction of immune tolerance rather than immunity. A number of observations demonstrate that antigen specific immune tolerance is the result of presentation of antigen within the liver. Firstly, allogeneic liver organ transplants are often well accepted by the recipient [1, 2] and lead to tolerance to further organ transplants from the same donor but not to third party grafts (“split tolerance”) [3]. Secondly, portal venous drainage of an allogeneic organ transplant [4–6] as well as pre-transplant portal venous injection of donor leukocytes lead to increased graft acceptance [7, 8]. Further evidence for tolerance induction after application of antigen via the portal route comes from the observation that porto-systemic shunting results in loss of tolerance towards orally ingested antigens [9]. It is important to note, that clonal elimination of antigen-reactive T cells or immune ignorance are not the mechanisms leading to immune tolerance in these situations, because adoptive transfer of lymphocytes from animals treated as described above again leads to development of antigen specific immune tolerance in the recipient [10]. Still, contribution of clonal elimination by apoptosis in the liver to hepatic tolerance induction can not be entirely excluded.
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The liver has a dual blood supply with portal venous blood draining together with hepatic arterial blood into the hepatic sinusoids. Leukocytes entering the liver with the bloodstream pass through the hepatic sinusoids and are obviously in direct contact with sinusoidal cells, the liver sinusoidal endothelial cells (LSEC) and the Kupffer cells. Intensive contact between sinusoidal cells and passenger leukocytes can occur for a number of reasons. The sinusoids are on average 5–7 μm wide forcing leukocytes to contact sinusoidal cells [11]; leukocyte margination occurs in hepatic sinusoids and together with intermittent blood flow and low perfusion pressure, contact of leukocytes with sinusoidal cells is enforced [12, 13]; leukocyte adhesion to LSEC does not require expression of selectins [14] but depends on constitutively expressed cellular adhesion molecules, such as CD54, CD106 and others.

Due to the fenestrated hepatic endothelium it was believed that hepatocytes are easily accessible for leukocytes passing through the liver and that this leads to direct and continuous immune surveillance of hepatocytes [15]. However, a recent publication has clearly shown that antigen presented exclusively by hepatocytes is not recognised by T cells specific for the antigen during their passage through the liver [16]. Moreover, it was demonstrated that blood borne particles ≥12 nm in diameter do not diffuse freely through the endothelial fenestrae (approx. diameter 100 nm) and do not have direct contact with receptors on the hepatocyte surface [17]. These studies reveal LSEC as a barrier that protects hepatocytes from direct contact with passenger leukocytes. Thus, Kupffer cells and especially LSEC, through repeated contact with passenger leukocytes, encounter all the prerequisites for influencing the immunological function of T cells. This becomes even more important as the entire blood volume is passed through the liver more than 300 times per day.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>LSEC</th>
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<tr>
<td>CD54</td>
<td>++</td>
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<td>CD102</td>
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<td>CD106</td>
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<td>CD14</td>
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<td>CD32</td>
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<td>CD36</td>
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<tr>
<td>MHC class I</td>
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<td>MHC class II</td>
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<td>CD80</td>
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<td>CD86</td>
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<td>CD40</td>
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<td>CD4</td>
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<td>CD11c</td>
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<td>CD95</td>
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<tr>
<td>CD95L</td>
<td>+</td>
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<td>TRAIL</td>
<td>+</td>
<td>n.d.</td>
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<tr>
<td>Membrane TNFα</td>
<td>*</td>
<td>n.d.</td>
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</table>

**++ strong expression, + expression, (+) faint expression, ø absent expression**

LSEC are microvascular endothelial cells and display a unique phenotype compared to macrovascular endothelial cells and microvascular endothelial cells from other organs. Table I summarises the molecules known to be expressed on LSEC as detected by flow cytometry, by functional in vitro assays or by immuno-histochemistry in liver sections. LSEC constitutively express molecules necessary for establishment of interaction with leukocytes, giving supportive evidence for the observation of constitutive leukocyte adhesion to LSEC in vivo. LSEC further express several pattern recognition receptors that enable them to act as scavenger cells (see below). Moreover, LSEC constitutively express co-stimulatory molecules (CD80, CD86, CD40) as well as MHC class I and II molecules necessary for presentation of antigen to T cells. Finally, LSEC express a number of molecules that are typically found on cells of myeloid origin such as CD4 and CD11c. It can be concluded from these observations that LSEC are endowed with a set of surface molecules that renders them competent for both, recruitment of T cells and antigen presentation to T cells.

However, LSEC in adult mice are not derived from the bone marrow (A. Limmer and P. Knolle, unpublished observation) whereas Kupffer cells continuously repopulate from the bone marrow [18]. It rather seems that LSEC regenerate from cells resident to the liver, which is not surprising given the haematopoietic function of the liver early in life and the capacity of transplanted livers to establish micro-chimerism [19, 20].
Scavenger function of liver LSEC

Receptor-mediated endocytosis of macromolecules is well studied in LSEC and known to occur with very high efficiency. The expression of pattern recognition receptors by LSEC, such as the mannose and scavenger receptors, points to a contribution of these cells to clearing sinusoidal blood of macromolecules that are recognised as “unwanted”, eg, by their glycosylation pattern or even low affinity receptor binding. LSEC are further endowed with the capacity to phagocytose particles up to 200 nm in diameter [21]. These findings imply that LSEC are part of the non-specific arm of the immune system.

However, there appears to be a conceptual problem with highly efficient antigen uptake by LSEC. What is the fate of ingested material? Are antigens only processed and presented by LSEC or are endocytosed antigens transported through the LSEC and handed over to hepatocytes? It has been shown that delivery of endocytosed antigens to the lysosomal compartment occurs comparatively slowly and in an inefficient way in LSEC [22]. How can LSEC combine efficient uptake with slow removal of ingested material via the lysosomal pathway? Tavassoli et al. have described that LSEC have the ability to transport endocytosed molecules in a vector fashion from the luminal side towards the hepatocytes and thereby accomplish transcytosis [23, 24]. This implies that clearance function of the liver is supported by efficient uptake of macromolecules by LSEC from the blood and shuttling of these molecules to the hepatocytes for excretion via the bile or metabolism. Although difficult to prove, transcytotic transport in LSEC has been assumed by independent groups to play a role in physiological clearance of macromolecules [17] as well as during the early steps of infection with hepatotropic viruses. However, we have evidence that antigen uptake by LSEC in vivo is accompanied by antigen presentation to T cells in vivo suggesting that at least part of the endocytosed antigen is used for presentation (see below).

LSEC present antigen on MHC II molecules to CD4+ T cells

Given the constitutive expression of costimulatory and MHC class II molecules together with the efficient uptake of antigen it is not surprising that LSEC present soluble antigens to CD4+ T cells. It is important to note that LSEC do not require a maturation step to induce antigen specific proliferation and cytokine release by antigen-specific CD4+ T cells. LSEC are almost as efficient as Kupffer and bone marrow derived “professional” antigen presenting cells with regard to stimulation of CD4+ T cells [25]. This finding raises the question of how hepatic immune tolerance relates to the unique function of LSEC to present antigen efficiently to CD4+ T cells. Antigen presentation by LSEC to CD4+ T cells is stringently controlled by mediators present in the local microenvironment such as PGE2 and IL-10 [29], which are expressed by other hepatic cell populations, eg, Kupffer cells [26–28].

However, not only soluble mediators released by neighbouring Kupffer cells but also portal blood constituents directly influence antigen presentation by LSEC. Endotoxin is physiologically present in portal venous blood at 100 pg/ml to 1 ng/ml [30] and is cleared by Kupffer cells as well as LSEC from the blood [31]. Pre-treatment of LSEC with endotoxin (in physiological concentrations) reduced antigen presentation to CD4+ T cells considerably [32].

Moreover, LSEC like dendritic cells can prime naïve CD4+ T cells, but unlike dendritic cells LSEC induce immune tolerance in CD4+ T cells upon cognate interaction. CD4+ T cells stimulated by antigen presenting LSEC show a regulatory phenotype characterised by the expression of IL-4 and IL-10 upon antigen-specific restimulation [33]. Again, LSEC do not require maturation in order to perform their antigen presenting function for naïve CD4+ T cells and thus are clearly different from microvascular endothelial cells from other organs [33–38]. We conclude that LSEC do not promote differentiation of naïve CD4+ T cells towards Th1 but rather induce regulatory T cells and thereby contribute to induction of hepatic immune tolerance.

Presentation of exogenous antigens on MHC class I molecules to CD8+ T cells

Functioning as scavenger cells for blood borne molecules LSEC are in a strategic position to present systemically distributed antigens to T cells. While MHC class II restricted presentation of exogenous antigens to CD4 T cells is well accepted, MHC class I restricted presentation of endocytosed antigens to CD8 T cells was believed to be restricted to dendritic cells and to occur only in
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Induction of CD8 T cells tolerance by cross-priming LSEC

LSEC not only cross-present antigen to a T cell hybridoma, but are also able to induce cytokine expression and proliferation in naive CD8+ T cell receptor transgenic T cells. The outcome of T cell priming by cross-presenting LSEC is clearly distinct from T cell priming by splenocytes or bone marrow derived antigen-presenting cells. T cells primed by LSEC lose their ability to express cytokines such as IFNγ and IL-2 and do not exhibit specific cytotoxicity any more compared to T cells primed by conventional antigen presenting cells. In vivo, LSEC induce antigen-specific (ovalbumin) CD8+ T cell tolerance as shown by inability of CD8+ T cells to reject an s.c. implanted syngeneic tumour expressing ovalbumin [42].

Several laboratories have shown that myeloid cross-presenting cells are required for induction of a protective CD8+ immune response to circulating soluble antigens. Our experiments add a new perspective to the way the organism co-ordinates the immune response to soluble antigens. CD8+ T cell immunity has not been achieved to soluble antigen unless antigens were added together with an adjuvant or were applied as particulate antigen. It has further remained unknown why intravenous injection of antigens results in specific immune tolerance. Efficient uptake and cross-presentation of soluble antigen by LSEC accompanied by induction of immune tolerance in CD4+ as well as CD8+ T cells may provide an explanation to these questions. Several of our experimental findings further support this view: (i) LSEC are 1.00 to 10.000 fold more efficient in cross-presentation of soluble antigen than has been reported for dendritic cells and macrophages, (ii) antigen uptake and subsequent cross-presentation is not enhanced by incorporation of antigen into immune complexes, whereas this process increases cross-presentation in dendritic by a factor of 1000, (iii) cross-presentation by LSEC occurs within 30 minutes after antigen contact.

LSEC-mediated tolerance in CD4+ and CD8+ T cells may protect the organism from an unwanted immune reaction towards antigens derived from the gastrointestinal tract and confine at the same time immune reactions once antigen circulates in the systemic blood pool. Antigenaemia is dangerous for the integrity of the organism as...
widespread activation of the immune system, as observed during sepsis, leads to breakdown of immunological function and can cause severe damage to vital organs by non-specific immune amplification mechanisms. It is therefore mandatory for immune homeostasis to restrict immune responses to antigen, which is present systemically. This scenario has been observed in vivo when functionally inactive virus specific T cells were detected exclusively in the liver of mice three days after onset of symptomatic influenza-induced pneumonia and after viraemia had occurred [43]. In addition to rendering T cells tolerant, clonal elimination by apoptosis may be operative in the liver as has been suggested by N. Crispe et al. Also here, sinusoidal cells appear to participate in this process as LSEC express death-inducing receptors (see Table I) and both LSEC and Kupffer cells induce apoptosis in susceptible T cells [45, 46]. It is intriguing to speculate that so far aetiologically undefined conditions with immune mediated hepatic damage, such as certain forms of autoimmune hepatitis, may be related to inefficient induction of immune tolerance through LSEC.

In contrast, presence of a “danger” signal such as an adjuvant, antigen in particulate form (resembling a pathogenic microorganism for phagocytosing cells) and coating of antigen with antibodies will result in improved antigen uptake and maturation of conventional myeloid antigen presenting cells shifting the immune response towards induction of CD8+ T cell immunity. Therefore, antigen formulation itself may, through antigen presentation on different antigen presenting cells, contribute to the “decision” whether immunity or tolerance is induced.

Conclusions

Two immune tolerance phenomena have been observed, but have not been sufficiently explained so far: hepatic tolerogenicity and induction of immune tolerance towards soluble systemically circulating antigens. Experimental data suggest that liver sinusoidal endothelial cells (LSEC) may represent a link between both phenomena for the following reasons: (i) LSEC are resident cells, lining liver sinusoids and have therefore direct contact to blood borne antigens, (ii) LSEC express various scavenging receptors rendering them very efficient in antigen uptake, (iii) LSEC express all known co-stimulatory molecules needed to stimulate T cells, (iv) LSEC can prime naive CD4+ T cells to become regulatory T cells, (v) LSEC cross-present exogenous antigens and render CD8+ T cells tolerant.

According to our model, the liver is “in charge” of tolerance induction towards antigens, which circulate in the blood or are released from hepatocytes. Among these antigens are self- and food antigens, against which an immune response would be deleterious. In contrast, immunity is observed when antigens are present locally in the periphery (ie, skin), when present only for a short time or when presented in the context of a danger signal (with adjuvant). The “decision” whether immunity or tolerance is induced, seems to require the participation of two different antigen presenting cell populations and different microenvironments. While induction of immunity requires activated dendritic cells, which are the best known immune stimulatory cells, and the unique well structured microenvironment of secondary lymphoid tissue, induction of immune tolerance in the liver appears to involve LSEC combined with the unique hepatic microenvironment.

Although in many aspects LSEC and dendritic cells share a common phenotype and the capacity to present exogenous antigen via MHC class I and II, there are important differences between LSEC and dendritic cells that may account for their different functional activity with regard to immune regulation. However, the molecular mechanisms underlying the “decision” whether immunity or immune tolerance is induced, still remain unknown. We conclude that LSEC represent a new type of antigen presenting cell that is organ-resident, does not require functional maturation and mediates down regulation rather than induction of immunity in the context of the local microenvironment. Our concept of local hepatic immune regulation explains a so far poorly recognised function of the liver: control of immunity by local induction of immune tolerance through tolerogenic organ-resident antigen-presenting cells.

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References

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10 Gorczyński RM. Adoptive transfer of unresponsiveness to allogeneic skin grafts with hepatic gamma delta T cells. Immunology 1994;81:27–35.


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