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Review

Liver Sinusoidal Endothelial Cells: a New Type of Organ-Resident Antigen-Presenting Cell

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Abstract. The induction of peripheral immune tolerance in the liver is a well-known phenomenon that is operative in different situations, such as tolerance to organ transplants and tolerance to oral antigens. The mechanisms leading to peripheral immune tolerance in the liver are still incompletely understood. While different cell populations of the liver have been implicated in and probably contribute in concert to the induction of hepatic immune tolerance, one hepatic cell type in particular seems to be suited for tolerance induction: liver sinusoidal endothelial cell (LSEC). LSEC are microvascular endothelial cells with a unique phenotype reminiscent of dendritic cells and a unique function as antigen-presenting cells for $CD4^+$ T cells. The hepatic microenvironment, i.e. portal venous constituents and soluble mediators from sinusoidal cell populations, tightly control antigen presentation by LSEC to avoid immune-mediated damage. LSEC, in contrast to other endothelial cells, have the capacity to prime naive $CD4^+$ T cells and induce cytokine release. Importantly, naive $CD4^+$ T cells primed by antigen-presenting LSEC differentiate into regulatory T cells, whereas T cells primed by bone marrow-derived professional antigen presenting cells differentiate into Th1 cells. Thus, LSEC represent a new type of organ-resident "non-professional" antigen-presenting cell that appears to be involved in the local control of the immune response and the induction of immune tolerance in the liver.

Key words: local immune response; antigen presentation; immune tolerance.

Immune Tolerance in the Liver

The liver seems to favor 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. First, allogeneic liver organ transplants are often well accepted by the recipient[6, 7] and lead to tolerance to further organ transplants from the same donor, but not to third party grafts ("split tolerance")[9]. Second, portal venous drainage of an allogeneic organ transplant[2, 4, 13] as well as pretransplant portal venous injection of donor leukocytes lead to increased graft acceptance[11, 27]. Further evidence for tolerance induction after application of antigen via the portal route comes from the observation that portosystemic shunting results in a loss of tolerance towards orally ingested antigens[39]. 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 situ-

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ations, because adoptive transfer of lymphocytes from animals treated as described above again leads to development of antigen-specific immune tolerance in the recipient[12].

Microanatomy of the Liver

Blood enters the liver via two separate ways: from the gastrointestinal tract via the portal vein and from the systemic circulation via the hepatic artery. After ramification, the portal vein drains together with the branches of the hepatic artery, into the hepatic sinusoids, leading to a mixed arterial-venous blood perfusion of the liver[37]. The sinusoids are formed by a unique population of endothelial cells, the liver sinusoidal endothelial cells (LSEC), which are fenestrated and lack a basement membrane, but, nevertheless, form a barrier between cells in the sinusoidal lumen and the hepatocytes[36]. Further cell populations of the hepatic sinusoid are the Kupffer cells and the liver-associated lymphocytes. Stellate cells are found in between the LSEC and hepatocytes in the space of Dissé.

Due to the fenestrated endothelium, it was believed that hepatocytes are easily accessible to leukocytes passing through the liver and that this leads to a direct and continuous immune surveillance of hepatocytes[1]. However, a recent publication has clearly shown that antigen presented exclusively by hepatocytes is not seen by T cells specific for this antigen during their passage through the liver[23]. Moreover, it was demonstrated that blood-borne particles ≥12 nm in diameter do not diffuse freely through the endothelial fenestrae and do not have direct contact with receptors on the hepatocyte surface[16]. These studies reveal LSEC as a barrier that protects hepatocytes from direct contact with passenger leukocytes.

Interaction of Passenger Leukocytes with Sinusoidal Cells of the Liver

It is evident that leukocytes entering the liver with the blood can interact with the sinusoidal lining cells of the liver, i.e. the Kupffer cells and the LSEC. The microcirculation in the liver has several unique characteristics: 1) the blood flow is discontinuous and chaotic (as migrating Kupffer cells can lead to flow arrest in downstream sinusoids by blocking the lumen)[26]; 2) flow velocity in the sinusoids is low enough to allow the establishment of leukocyte-endothelial interaction[28]; 3) there is constitutive interaction between LSEC and leukocytes in the physiological situation[38]. Although leukocyte adhesion to endothelial cells in general is thought to require the expression of selectins on the endothelial cells, expression of selectins is neither necessary for leukocyte-LSEC contact in the liver nor is expression of selectins detected on LSEC[38]. At the present time it is still unclear why there is constitutive leukocyte--LSEC interaction in the physiological situation.

Phenotype of LSEC

The sinusoidal endothelial cells of the liver have a unique phenotype compared with both the macrovascular and microvascular endothelial cells from other organs. Table 1 summarizes the molecules known to be expressed on LSEC as detected by flow cytometry on isolated LSEC or by immunohistochemistry *in situ*. The molecules necessary for the interaction of LSEC with leukocytes are constitutively expressed at high levels, which provides supportive evidence for constitutive leukocyte-LSEC adhesion observed *in vivo*. LSEC constitutively express all costimulatory molecules as well as the MHC class I and II molecules necessary for the presentation of antigen to T cells[21, 24]. The expression level of these molecules, however, is much lower than in so-called professional antigen-presenting cells such as dendritic cells. LSEC further express a number of molecules that are normally found on bone marrow--derived cells such as CD4[33] and CD11c[20]. However, LSEC are not derived from the bone marrow, but, following partial hepatectomy, apparently regenerate from resident liver cells (KNOLLE and ARNOLD, unpublished observation). 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.

Table 1. Phenotype of liver sinusoidal endothelial cells (LSEC)

	LSEC	Endothelial cells	Inducible by IFN-γ
CD54	+++	+	++
CD106	–	–	(+)
CD62E	–	–	(+)
CD31	++	++	
Mannose receptor	+++	–	
Scavenger receptor	+++	–	
MHC-I	+++	++	
MHC-II	+	–	(+)
CD80	+	–	(+)
CD86	+	–	(+)
CD40	++	+	
CD11c	+	–	
CD4	+	–	

Antigen Presentation by LSEC to CD4$^+$ T Cells

We have studied the capacity of LSEC to function as antigen-presenting cells for CD4$^+$ T cells using pure primary cultures of LSEC from mice. Using primary cell cultures of pure LSEC and cloned antigen-specific CD4$^+$ T cells, we have shown that LSEC present nominal antigen to CD4$^+$ T cells[24]. It is important to note that LSEC do not need to undergo maturation and do not require exogenous cytokines such as interferon γ (IFN-γ) to present antigen efficiently to CD4$^+$ T cells. LSEC are almost as efficient in presenting antigen to CD4$^+$ T cells as Kupffer cells and bone marrow-derived antigen-presenting cells[24]. This implies that LSEC may present antigen to CD4$^+$ T cells in the liver.

Regulation of Antigen Presentation in LSEC

Regulation of T cell activation in the liver is very important. Uncontrolled activation of T cells in the liver is detrimental and leads to severe liver damage, as shown in the model of T cell-mediated liver damage induced by intravenous injection of the lectin concanavalin A (ConA)[34]. Upon binding of ConA, LSEC activate CD4$^+$ T cells, which leads to the destruction of LSEC by the activated T cells and, consequently, to hepatocyte damage[17]. Therefore, antigen presentation by LSEC must be stringently controlled in order to avoid unnecessary activation of T cells.

We have reported that Kupffer cells secrete a potent immuno-modulatory cytokine, interleukin 10 (IL-10), in response to physiological concentrations of endotoxin in the portal venous blood[19, 22]. In a paracrine fashion, IL-10 released from Kupffer cells efficiently downregulates antigen presentation by LSEC through two mechanisms: downregulation of receptor-mediated antigen uptake in LSEC and downregulation of the costimulatory molecules CD80 and CD86 as well as MHC class II molecules[21]. Antigen presentation in LSEC is further regulated by endogenously produced prostaglandins (KNOLLE, unpublished observation) which are generated in response to physiological constituents of portal venous blood. Furthermore, endotoxin itself appears to have a direct negative influence on LSEC for antigen presentation to CD4$^+$ T cells. Physiological concentrations of endotoxin downregulate antigen presentation to CD4$^+$ T cells by more than 50% only in LSEC but not in bone marrow-derived antigen-presenting cells[18]. The endotoxin effect on LSEC is mediated by alkalization of the endosomal compartment, which prevents endosomal enzymatic proteolysis, and by downregulation of MHC class II molecules as well as costimulatory molecules[18]. These findings may explain why antigens derived from the gastrointestinal tract, which reach the liver together with endotoxin, do not provoke an immune response in the liver. However, this does not explain the induction of immune tolerance. Therefore, we investigated the ability of LSEC to influence the function of T cells using naive CD4$^+$ T cells, which are still susceptible to regulation of T cell function, from T cell receptor transgenic animals.

Priming of Naive CD4$^+$ T Cells by Antigen-Presenting LSEC

The priming of naive CD4$^+$ T cells is believed to occur exclusively in bone marrow-derived, professional antigen-presenting cells such as dendritic cells or macrophages. Although LSEC are not myeloid cells they can present antigen to naive CD4$^+$ T cells[20]. Again, LSEC do not require exogenous cytokines such as IFN-γ to efficiently present antigen to naive CD4$^+$ T cells[20]. This feature distinguishes LSEC from the microvascular endothelial cells of other organs, which require prestimulation in order to present antigen to T cells[8, 25, 31, 35]. So far, the priming of naive T cells has been thought to occur exclusively in lymphatic tissue, but LSEC are organ-resident cells that do not migrate into lymphatic tissue. Thus, the liver appears to be a unique locus where T cell priming occurs outside lymphatic tissue.

Induction of Regulatory CD4$^+$ T Cells by LSEC

The local environment and costimulatory signals delivered from the antigen-presenting cell during the priming of naive CD4$^+$ T cells determine the functional phenotype of the T cell (Th1, Th2, Th3 or Tr). Th1 cells are required to mount protective cell-mediated immune responses against tumors or pathogens, whereas Th2 cells support the induction of antibody responses and, in certain situations, appear to counteract Th1 cell action. Th3 and Tr cells have been implicated in the induction of immune tolerance. We have demonstrated that CD4$^+$ T cells primed by antigen-presenting LSEC do not differentiate into Th1 cells. T cells primed by LSEC express the cytokines IL-4 and IL-10 upon restimulation, which is consistent with a Th0 phenotype of cytokine secretion[20]. In contrast, CD4$^+$ T cells primed by professional antigen-presenting cells develop into Th1 cells expressing large amounts of IFN-γ upon restimulation[20]. Importantly, T cells primed by LSEC can

suppress antigen-specific T cell responses, which points to a regulatory function of these T cells (KNOLLE, unpublished observation). Thus, the priming of naive T cells by antigen-presenting LSEC seems not to lead to immunity, but to the induction of immune tolerance.

Local Control of the Immune Response in the Liver

In the liver, blood-borne antigen is preferentially taken up by LSEC and not by other hepatic cell populations (KNOLLE and LIMMER, unpublished observation). Given the constitutive interaction of LSEC with leukocytes in the liver, it seems likely that antigen endocytosed by LSEC is presented to T cells in the liver and that the functional LSEC – T cell interaction occurs *in vivo*. LSEC may represent a new type of antigen-presenting cell that operates in a peripheral organ to promote immune surveillance on the one hand, but rather induces peripheral immune tolerance to ensure organ integrity on the other.

However, it is unlikely that LSEC are the only cell population involved in peripheral immune-tolerance induction in the liver. Almost every cell population of the liver has been demonstrated to be implicated in tolerance induction: Kupffer cells[5, 32] and a specific subset of hepatic dendritic cells in the portal field[14] appear to contribute to tolerance against alloantigens. Immune tolerance in the liver is likely to be achieved by a combination of mechanisms such as clonal deletion and immune deviation. The liver is known for its ability to eliminate apoptotic T cells[15] and it is an open question whether apoptosis of T cells is even induced in the liver. Hepatocytes[3, 10], as well as LSEC and Kupffer cells[29, 30], are reported to have the capacity to induce apoptosis in susceptible T cells. However, at present it is still unclear whether induction of apoptosis in T cells contributes to immune tolerance in the liver.

References

1. ANDO K., GUIDOTTI L. G., CERNY A., ISHIKAWA T. and CHISARI F. V. (1994): CTL access to tissue antigen is restricted *in vivo*. J. Immunol., **153**, 482–488.
2. BARKER C. F. and CORRIERE J. N. JR. (1967): Canine renal homotransplantation with venous drainage via the portal vein. Ann. Surg., **165**, 279–282.
3. BERTOLINO P., TRESCOL-BIEMONT M. C. and RABOURDIN-COMBE C. (1998): Hepatocytes induce functional activation of naive $CD8^+$ T lymphocytes but fail to promote survival. Eur. J. Immunol., **28**, 221–236.
4. BOECKX W., SOBIS H., LACQUET A., GRUWEZ J. and VANDEPUTTE M. (1975): Prolongation of allogeneic heart graft survival in the rat after implantation on portal vein. Transplantation, **19**, 145–149.
5. CALLERY M. P., KAMEI T. and FLYE M. W. (1989): Kupffer cell blockade inhibits induction of tolerance by the portal venous route. Transplantation, **47**, 1092–1094.
6. CALNE R. Y. (1969): Induction of immunological tolerance by porcine liver allografts. Nature, **223**, 472–476.
7. CANTOR H. M. and DUMONT A. E. (1967): Hepatic suppression of sensitization to antigen absorbed into the portal system. Nature, **215**, 744–745.
8. CUNNINGHAM A. C., ZHANG J. G., MOY J. V., ALI S. and KIRBY J. A. (1997): A comparison of the antigen-presenting capabilities of class II MHC-expressing human lung epithelial and endothelial cells. Immunology, **91**, 458–463.
9. DAHMEN U., QIAN S., RAO A. S., DEMETRIS A. J., FU F., SUN H., GAO L., FUNG J. J. and STARZL T. E. (1994): Split tolerance induced by orthotopic liver transplantation in mice. Transplantation, **58**, 1–8.
10. GALLE P. R., HOFMANN W. J., WALCZAK H., SCHALLER H., OTTO G., STREMMEL W., KRAMMER P. H. and RUNKEL L. (1995): Involvement of the CD95 (APO-1/Fas) receptor and ligand in liver damage. J. Exp. Med., **182**, 1223–1230.
11. GORCZYNSKI R. M. (1992): Immunosuppression induced by hepatic portal venous immunization spares reactivity in IL-4 producing T lymphocytes. Immunol. Lett., **33**, 67–77.
12. GORCZYNSKI R. M. (1994): Adoptive transfer of unresponsiveness to allogeneic skin grafts with hepatic $\gamma\,\delta^+$ T cells. Immunology, **81**, 27–35.
13. GORCZYNSKI R. M., CHAN Z., CHUNG S., COHEN Z., LEVY G., SULLIVAN B. and FU X. M. (1994): Prolongation of rat small bowel or renal allograft survival by pretransplant transfusion and/or by varying the route of allograft venous drainage. Transplantation, **58**, 816–820.
14. GORCZYNSKI L., CHEN Z., HU J., KAI Y., LEI J., RAMAKRISHNA V. and GORCZYNSKI R. M. (1999): Evidence that an OX-2-positive cell can inhibit the stimulation of type 1 cytokine production by bone marrow-derived B7-1 (and B7-2)-positive dendritic cells. J. Immunol., **162**, 774–781.
15. HUANG L., SOLDEVILA G., LEEKER M., FLAVELL R. and CRISPE I. N. (1994): The liver eliminates T cells undergoing antigen-triggered apoptosis *in vivo*. Immunity, **1**, 741–749.
16. KEMPKA G. and KOLB-BACHOFEN V. (1988): Binding, uptake, and transcytosis of ligands for mannose-specific receptors in rat liver: an electron microscopic study. Exp. Cell Res., **176**, 38–48.
17. KNOLLE P. A., GERKEN G., LOSER E., DIENES H. P., GANTNER F., TIEGS G., MEYER ZUM BUSCHENFELDE K. H. and LOHSE A. W. (1996): Role of sinusoidal endothelial cells of the liver in concanavalin A-induced hepatic injury in mice. Hepatology, **24**, 824–829.
18. KNOLLE P. A., GERMANN T., TREICHEL U., UHRIG A., SCHMITT E., HEGENBARTH S., LOHSE A. W. and GERKEN G. (1999): Endotoxin down-regulates T cell activation by antigen-presenting liver sinusoidal endothelial cells. J. Immunol., **162**, 1401–1407.
19. KNOLLE P., SCHLAAK J., UHRIG A., KEMPF P., MEYER ZUM BUSCHENFELDE K. H. and GERKEN G. (1995): Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. J. Hepatol., **22**, 226–229.

20. KNOLLE P., SCHMITT E., JIN S., GERMANN T., DUCHMANN R., HEGENBARTH R., GERKEN G. and LOHSE A. (1999): Liver sinusoidal endothelial cells can prime naive $CD4^+$ T cells in the absence of IL-12 and induce IL-4 production in primed $CD4^+$ T cells: Implications for tolerance induction in the liver. Gastroenterology, **116**, 1428–1440.
21. KNOLLE P. A., UHRIG A., HEGENBARTH S., LOSER E., SCHMITT E., GERKEN G. and LOHSE A. W. (1998): IL-10 down-regulates T cell activation by antigen-presenting liver sinusoidal endothelial cells through decreased antigen uptake via the mannose receptor and lowered surface expression of accessory molecules. Clin. Exp. Immunol., **114**, 427–433.
22. KNOLLE P. A., UHRIG A., PROTZER U., TRIPPLER M., DUCHMANN R., MEYER ZUM BUSCHENFELDE K. H. and GERKEN G. (1998): Interleukin-10 expression is autoregulated at the transcriptional level in human and murine Kupffer cells. Hepatology, **27**, 93–99.
23. LIMMER A., SACHER T., ALFERINK J., KRETSCHMAR M., SCHONRICH G., NICHTERLEIN T., ARNOLD B. and HAMMERLING G. J. (1998): Failure to induce organ-specific autoimmunity by breaking of tolerance: importance of the microenvironment. Eur. J. Immunol., **28**, 2395–2406.
24. LOHSE A. W., KNOLLE P. A., BILO K., UHRIG A., WALDMANN C., IBE M., SCHMITT E., GERKEN G. and MEYER ZUM BUSCHENFELDE K. H. (1996): Antigen-presenting function and B7 expression of murine sinusoidal endothelial cells and Kupffer cells. Gastroenterology, **110**, 1175–1181.
25. MA W. and POBER J. S. (1998): Human endothelial cells effectively costimulate cytokine production by, but not differentiation of, naive $CD4^+$ T cells. J. Immunol., **161**, 2158–2167.
26. MACPHEE P. J., SCHMIDT E. E. and GROOM A. C. (1995): Intermittence of blood flow in liver sinusoids, studied by high-resolution *in vivo* microscopy. Am. J. Physiol., **269**, G692–698.
27. MAY A. G., BAUER S., LEDDY J. P., PANNER B., VAUGHAN J. and RUSSELL P. S. (1969): Survival of allografts after hepatic portal venous administration of specific transplantation antigen. Ann. Surg., **170**, 824–832.
28. MCCUSKEY R. S. and REILLY F. D. (1993): Hepatic microvasculature: dynamic structure and its regulation. Semin. Liver Dis., **13**, 1–12.
29. MÜSCHEN M., WARSKULAT U., DOUILLARD P., GILBERT E. and HÄUSSINGER D. (1998): Regulation of CD95 (APO-1/Fas) receptor and ligand expression by lipopolysaccharide and dexamethasone in parenchymal and nonparenchymal rat liver cells. Hepatology, **27**, 200–208.
30. MÜSCHEN M., WARSKULAT U., PETERS-REGEHR T., BODE J. G., KUBITZ R. and HÄUSSINGER D. (1999): Involvement of CD95 (Apo-1/Fas) ligand expressed by rat Kupffer cells in hepatic immunoregulation. Gastroenterology, **116**, 666–677.
31. RISAU W., ENGELHARDT B. and WEKERLE H. (1990): Immune function of the blood-brain barrier: incomplete presentation of protein (auto-)antigens by rat brain microvascular endothelium *in vitro*. J. Cell. Biol., **110**, 1757–1766.
32. ROLAND C. R., MANGINO M. J., DUFFY B. F. and FLYE M. W. (1993): Lymphocyte suppression by Kupffer cells prevents portal venous tolerance induction: a study of macrophage function after intravenous gadolinium. Transplantation, **55**, 1151–1158.
33. SCOAZEC J. Y. and FELDMANN G. (1990): Both macrophages and endothelial cells of the human hepatic sinusoid express the CD4 molecule, a receptor for the human immunodeficiency virus. Hepatology, **12**, 505–510.
34. TIEGS G., HENTSCHEL J. and WENDEL A. (1992): A T cell-dependent experimental liver injury in mice inducible by concanavalin A. J. Clin. Invest., **90**, 196–203.
35. VORA M., YSSEL H., DE VRIES J. E. and KARASEK M. A. (1994): Antigen presentation by human dermal microvascular endothelial cells. Immunoregulatory effect of IFN-γ and IL-10. J. Immunol., **152**, 5734–5741.
36. WISSE E. (1970): An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. J. Ultrastruct. Res., **31**, 125–150.
37. WISSE E., DE ZANGER R. B., CHARELS K., VAN DER SMISSEN P. and MCCUSKEY R. S. (1985): The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Dissé. Hepatology, **5**, 683–692.
38. WONG J., JOHNSTON B., LEE S. S., BULLARD D. C., SMITH C. W., BEAUDET A. L. and KUBES P. (1997): A minimal role for selectins in the recruitment of leukocytes into the inflamed liver microvasculature. J. Clin. Invest., **99**, 2782–2790.
39. YANG R., LIU Q., GROSFELD J. L. and PESCOVITZ M. D. (1994): Intestinal venous drainage through the liver is a prerequisite for oral tolerance induction. J. Pediatr. Surg., **29**, 1145–1148.

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