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Liver sinusoidal lymphocytes: their immune functions

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Abstract Recent studies strongly suggest that the liver plays an important immunoregulatory role. Evidence of its role in general immune responsiveness originates from observation that, in recipients of liver grafts, the survival of other allografts is significantly prolonged. The question arises as to which blood lymphocyte subsets, most likely to be responsible for this phenomenon, marginate in liver sinusoids. To study this problem, a liver ex vivo perfusion model was designed for rats. In situ W/WAG livers were washed clear of sinusoidal marginating cells prior to and after 1 h perfusion with syngeneic blood. The number of blood cells retained in liver sinusoids, their phenotypes, the responsiveness to mitogen (PHA, 90 μg/ml) and cytotoxicity against YAC-1 tumour cells were examined. Our studies showed that rat liver retains in the sinusoids a population of blood cells, enriched in NK, $CD8^+$ and MHC class II^+ cells, displaying a high cytotoxic activity and low responsiveness to mitogen stimulation, with a capacity of about 10^6 cells/g of tissue.

Key words Liver transplantation · Sinusoidal lymphocytes · Tolerance

Introduction

Recent studies from our and other laboratories strongly suggest that the liver plays an important immunoregulatory role in general and local immunity [2, 4, 6, 7, 9, 10, 12–16, 21, 22]. Evidence of its role in general immune responsiveness originates from the observation that in liver recipients who do not receive any immunosuppressive treatment, the survival of heart, skin and kidney allografts is significantly prolonged [1, 2, 6, 14]. Moreover, there are numerous examples of survival of liver grafts in man after the withdrawal of immunosuppressive treatment [1, 19, 21]. These data suggest that the role of the liver is not limited to elimination and inactivation of damaged cells and digestive and bacterial antigens, but also encompasses the immune responsiveness to non-self antigens. The main cell mediators in these reactions are lymphocytes. It appears that the local immune function of the liver is associated with a specific population of lymphocytes, most probably of blood origin, which are transiently retained in the liver [10]. These cells, marginating in hepatic sinusoids, appear to be responsible for the destruction of malignant cells reaching the liver via the bloodstream [3, 9, 22]. The question arises as to which blood lymphocyte subsets marginate in liver sinusoids. Since the in vivo studies on trapping of lymphocytes in liver sinusoids are difficult because of other functioning lymphatic organs (mainly the spleen) actively halting circulating lymphocytes, we decided to carry out studies on an extracorporeal liver perfusion model. The study was devoted to the investigation of the process of halting circulating blood lymphocytes in the liver sinusoids and the characterization of the phenotypes of these cells.

Materials and methods

Animals

Wistar (W/Wag) (RT1u) rats with body weights of 200–250 g were used.

Liver perfusion

The rat liver perfusion system consisted of a peristaltic pump, an oxygenator with 0.1 m^2 gas exchange surface, and a heat exchanger (Fig. 1). The temperature of 37 °C was maintained in the perfusion circuit by means of a water thermostat. The rats were heparinized and exsanguinated. Their livers were washed out cells through the portal vein with 40 ml of PBS and the collected wash-out cells isolated by centrifugation at 1600 rpm for 35 min on a Lymphoprep gradient (Nyegaard, Oslo, Norway) and collected for further studies. Subsequently, in situ perfusion of the liver was carried out for 1 h with syngeneic blood diluted 1 : 2 with PBS. After completion of perfusion, the liver vasculature was again washed out as described above and the collected cells were isolated.

Parameters measured

The following parameters were measured:
1. The number of blood cells washed out from the liver sinusoids
2. Phenotypical characteristics of cells using monoclonal antibodies. For immunohistochemical staining, the indirect alkaline phosphatase anti-alkaline phosphatase (APAAP) technique was applied. Cytospines fixed in acetone were incubated in normal rabbit serum (NRS), followed by the application of primary mouse MoAbs: W3/13 (CD3) – pan T cells, W3/25 (CD4) – helper/inducer cells, OX6 – MHC class II antigens bearing cells, OX8 (CD8) – suppressor/cytotoxic cells, OX19 (CD5) – T cells, 3.2.3 – NK cells (Serotec, UK). The secondary and tertiary antibodies consisted of rabbit-anti-mouse immunoglobulins and APAAP complex. All incubations were carried out at room temperature for 30 min, followed by a wash in two changes of TBS for 10 min. The reaction product was developed by incubation with chromogenic alkaline phosphatase substrate for 15 min. Then 400 cells were counted with light microscope.
3. Responsiveness of washed out cells to mitogen (PHA, 90 μg/ml, Wellcome, HA15). Microcultures containing 1×10^5/well cells suspended in medium RPMI 1640 + 15 % FCS were cultured with phytohemaglutinin (final mitogen concentration 10 μg/ml and 2 μg/ml) for 72 h. Proliferation was then measured with an 18-h pulse of [^{3}H]thymidine and expressed as the number of counts per minute (cpm).
4. Cytotoxicity of washed out cells against YAC-1 tumour cells. Target cells (3×10^6) were labelled for 60 min at 37 °C using 100 μCi of sodium chromate (Amersham, UK) and resuspended in RPMI 1640 + 10 ml FCS to a concentration of 2.5×10^4 ml of lymphocytes. They were added to round-bottomed small culture tubes together with 0.2 ml of YAC-1 cells to make the effector-target ratio 20 : 1, 10 : 1 and 5 : 1. All tests were done in triplicate and incubated for 4 h at 37 °C in an atmosphere of 5 % CO_2 in air. After incubation, the tubes were centrifuged and 0.2 ml samples of supernatant were transferred to the other tubes and both aliquots counted on a gamma counter. The data were expressed as the percentage of ^{51}Cr release for each sample.

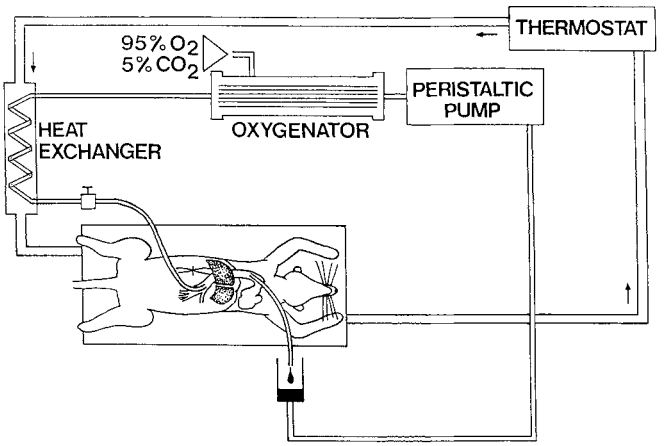

Fig. 1 The in situ ex vivo rat liver perfusion system

Table 1 Phenotypes of cells retrieved from liver sinusoids (median) ($n = 6$). Perfusion of liver with syngeneic blood in an extracorporeal circuit brought about reaccumulation in the sinusoids of similar populations of lymphocytes as had been found prior to perfusion. Both, pre- and postperfusion populations differed from blood populations (* ss vs blood)

MoAb	Prior to perfusion •	After perfusion	Blood
W3/13	48.0	49.0	68.0
W3/25	36.6*	35.2*	49.7
OX6	32.0*	25.5*	21.7
OX8	33.0*	26.5*	24.5
OX19	28.0*	25.6*	58.2
3.2.3	25.3*	22.5*	10.0

Results

Number of blood cells retained in liver sinusoids

The number of cells retrieved from liver sinusoids before perfusion was $1.08 \pm 0.3 \times 10^6$/g of liver tissue and after perfusion it was $0.96 \pm 0.2 \times 10^6$/g of liver tissue.

Phenotypical characteristics of cells retained in liver sinusoids

The results of phenotypical characteristics of cells washed out from liver sinusoids and perfusing blood are presented in Table 1. Monoclonal antibody analysis showed a prevalence in the wash-out population before perfusion, as compared to the perfusing blood, of OX8$^+$ suppressor/cytotoxic (33 % vs 24.5 %, respectively), OX6$^+$ class II antigen$^+$ (32 % vs 21.7 %) and 3.2.3$^+$ NK cells (25.3 vs 10 %), whereas it contained fewer W3/25$^+$ helper/inducer (36.6 % vs 49.7 %) and OX19$^+$ T cells (28 % vs 58.2 %). Cells isolated from wash-out fluid af-

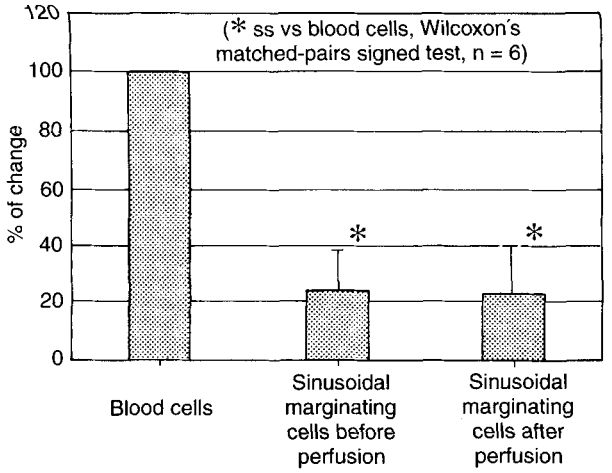

Fig.2 The pre- and postperfusion responsiveness to mitogen (PHA, 90 μg/ml) of the liver sinusoidal wash-out cells compared to perfusing blood cells

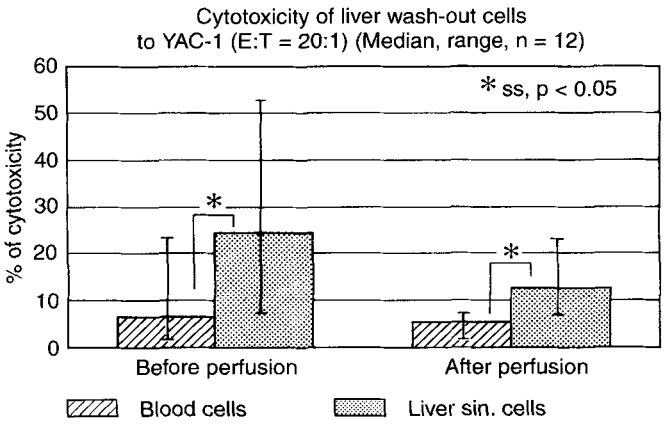

Fig.3 The pre- and postperfusion level of cytotoxicity against YAC-1 of the liver sinusoidal wash-out cells and perfusing blood cells

ter perfusion displayed the same characteristics as the population washed out before perfusion.

Responsiveness to PHA

The responsiveness of lymphocytes to PHA in the wash-out fluid before perfusion was significantly lower (mean 76.4 %) than of the perfusing blood lymphocytes and, similarly, the responsiveness of wash-out fluid after perfusion cells was also diminished (mean 77.2 %) (Fig. 2).

Cytotoxicity against YAC-1 tumour cells

The cytotoxicity against YAC-1 (E : T = 20 : 1) cells of the wash-out fluid cells before perfusion was 24.49 % (median values). It differed significantly from the cytotoxicity of cells in the perfusing blood, where it was 6.52 %. Cytotoxicity of postperfusion wash-out cells was 12.58 % and, again, it differed significantly compared to the cytotoxicity of cells in perfusing blood, where it was 5.43 % (Fig. 3).

Discussion

These studies provided the following information: (a) a liver perfused ex vivo with syngeneic blood retained its in vivo function of marginating mononuclear cells in sinusoidal blood, at a concentration of approximately 1 million cells/g of tissue, (b) perfusion of a liver with syngeneic blood brought about the reacummulation in the sinusoids of a similar population of lymphocytes as found prior to perfusion, and (c) the blood mononuclear cells retained in the liver sinusoids after perfusion showed the same low responsiveness to PHA, and a high level of cytotoxicity to YAC-1 tumour cells as the preperfusion population.

The trapping of cells with NK and suppressor/cytotoxic characteristics in an ex vivo perfused liver, demonstrated in these experiments, points to the capacity of the liver to select certain cell population from sinusoidal blood. It has been shown in the in vivo studies that certain populations of blood lymphocytes have a predilection to accumulate temporarily in the liver [10, 11, 12, 18]. However, the mechanism of this process remains unclear. Circulating lymphocytes that arrive at the liver must adhere to the endothelial cells in order either to extravasate or to carry out their in situ tasks in the sinusoids. The adhesion molecules are present both on mononuclear and endothelial cells. These proteins fall into two main groups: (a) adhesion proteins which are constitutionally expressed by all normal microvascular endothelial cells, such as ICAM-2 (CD102, intracellular adhesion molecule-2), and (b) inducible proteins, absent from normal endothelial cells but upregulated by inflammatory cytokines, such as ICAM-1 (CD-54, intracellular adhesion molecule-1), VCAM-1 (CD106, vascular cell adhesion molecule-1), and E-selectin (CD62E) [8, 20]. Significant differences between sinusoidal endothelial cells and microvascular endothelial cells were only observed for ICAM-1. In contrast to most microvascular endothelial cells in the body, sinusoidal endothelial cells display a constitutionally high expression of ICAM-1 [17]. Like the other capillary endothelial cells, sinusoidal endothelial cells express ICAM-2, but do not display detectable levels of VCAM-1 and E-selectin. However, VCAM-1 and E-selectin can be upreg-

ulated on sinusoidal endothelial cells in inflammatory conditions. Adhering lymphocytes possess on their surface ligands to the endothelial cell adhesion molecules as LFA-1 (CD18/CD11a, lymphocyte function antigen), VLA 4 (CD 49d, very late antigen 4) and CD44 [3, 17].

Which of these molecules participate in the process of margination of NK and other sinusoidal populations in the rat liver remains the subject of our investigations. Recent results of our studies suggest that LFA-1/ICAMs and VLA-4/VCAM-1 are the main pathways of the lymphocyte-endothelial adhesion process in liver sinusoids [5]. These interactions could be further modulated and potentiated by the presence of other cell surface molecules. Cells from liver sinusoids strongly express LFA-2 (CD2), for which the specific ligand LFA-3 (CD58) is constitutionally present on sinusoidal endothelial cells. The action of these molecules may represent an additional factor in the adhesion of lymphocytes to endothelial and Kupffer cells in the sinusoidal lumen.

Taken together, perfused rat liver selectively retains in the sinusoids a specific population of blood cells, enriched in NK, $CD8^+$ and MHC class II^+ cells, displaying a high cytotoxic activity and low responsiveness to mitogen stimulation.

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