Virchows Arch [Pathol Anat] (1986) 409:407–416

Virchows Archiv A

Hepatic vascular endothelial cells heterogenously express surface antigens associated with monocytes, macrophages and T lymphocytes

H. Nagura[1], T. Koshikawa[1], Y. Fukuda[2], and J. Asai[3]

[1] Research Institute for Disease Mechanism and Control,
[2] Department of Internal Medicine, and
[3] Department of Pathology, Nagoya University School of Medicine,
65 Tsurumai-cho, Showa-ku, Nagoya 466, Japan

Summary. During studies of the antigenic and functional properties of hepatic sinusoidal lining cells in situ, we found that only the sinusoidal endothelial cells share antigens with a peripheral blood macrophage subset capable of presenting soluble antigens and triggering autologous mixed lymphocyte reactions. They were HLA-DR+, OKM1−, OKM5+. Vascular endothelial cells in the portal areas and central veins were HLA-DR+, OKM1− and OKM5−. The sinusoidal endothelial cells also expressed an antigen found on helper/inducer (OKT4 and Leu3a) T lymphocytes. Thus, the present study suggests that endothelial cells in different anatomic compartments in the liver heterogenously express surface antigens associated with monocytes, macrophages and T lymphocytes and possess distinct immunological functions.

Key words: Endothelial cells – Monocyte – Liver – Immunohistochemistry – Monoclonal antibodies

Introduction

With the recent development of highly specific monoclonal antibodies to antigenic determinants on functionally distinct subpopulation of human monocytes/macrophages, their immune functions have been characterized in greater detail (Breard et al. 1980; Raff et al. 1980; Rosenberg et al. 1981; Nunez et al. 1982; Shen et al. 1983). Monoclonal antibodies OKM1 and OKM5, for example, can be used to isolate functionally distinct monocyte/macrophage subsets and clarify their role as antigen presenting cells (APC) in the autologous mixed lymphocyte reaction (AMLR) and in the presentation of soluble and cell surface antigens (Breard et al. 1980; Shen et al. 1983).

It is generally accepted that human vascular endothelial cells carry the HLA-DR antigen, are required for antigen presentation and are capable

Offprint requests to: H. Nagura at the above address

of substituting for macrophages in the lymphoproliferative response of the T cell to soluble protein antigens (Hirshberg et al. 1975; Moraes and Stastny 1977; Hirschberg et al. 1980; Nunez et al. 1983). Recently, in the immunohistochemical study using the OKM1 and OKM5, Knowels et al. (1984) have shown them to share antigens with the peripheral blood macrophage subset that posseses the capacity to present self antigens in AMLR. Moreover, the splenic sinusoidal cells express the cell surface antigens found on the helper/inducer and suppressor/cytotoxic T lymphocyte subsets (Buckley et al. 1985). Thus vascular endothelial cells may be important effector cells for immune responses. However, the antigenic and functional properties of sinusoidal lining cells of the liver have not been studied extensively. The sinusoids of the liver consist of Kupffer cells (KuC) and fat storing cells (Ito cells) as well as endothelial cells (EnC). The function of these sinusoidal lining cells range from participation in the mononuclear phagocyte system to acting as a non-phagocytic lining cells (Praaning-van Dalen et al. 1981; Nagura et al. 1985), but the question of which cells are responsible for antigen presentation has not been answered. In this study, we utilized several monoclonal antibodies to the surface antigens of a variety of cells to characterize the sinusoidal lining cells of the human liver, and discussed the distinctive surface phenotype and immunological functions of EnC in different anatomic compartments in the liver.

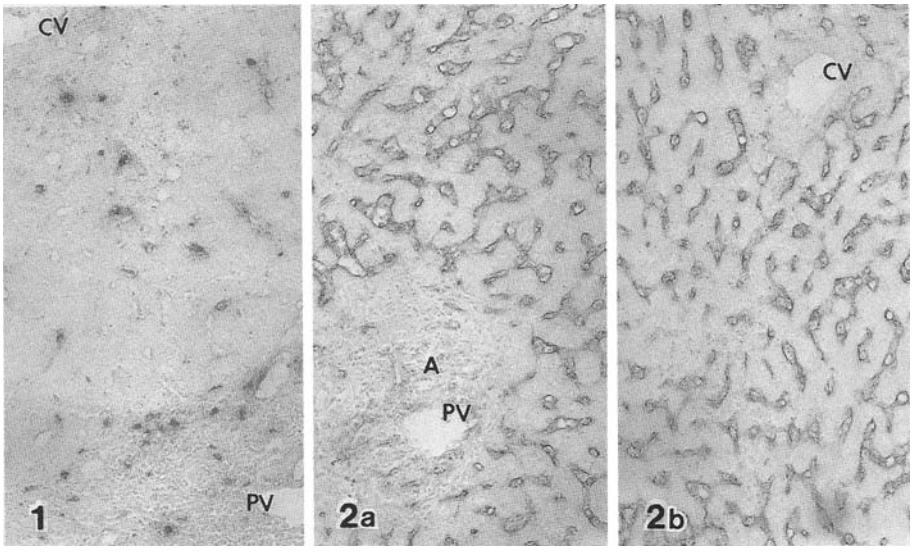
Materials and methods

Tissue specimens. Needle biopsy specimens were obtained by the Tru-cut needle technique from 38 patients with histologically proven chronic hepatitis (chronic aggressive hepatitis, 21 cases; chronic persistent hepatitis, 7 cases) and cirrhosis (postnecrotic cirrhosis, 4 cases; posthepatitic cirrhosis, 6 cases). Histologically normal tissue was also obtained by biopsy from three patients undergoing surgery for gastric cancer for diagnostic purposes. A part of each specimen was immediately fixed in periodate-lysine-paraformaldehyde (PLP) (McLean and Nakane 1974) for 5–6 h at 4° C, washed in increasing concentrations of sucrose in phosphate buffered saline (PBS), and finally placed in 20% sucrose. The fixed specimens were embedded in Tissue-Tek OCT compound (Miles Pharmaceutical, Naperville, Ill.), frozen in dry-ice ethanol, and sectioned (6–8μ) in a cryostat. The sections were placed on egg-albumin coated slides and dried in air.

Antibodies. OKM1, OKM5, OKT4 and OKT8 monoclonal antibodies (MoAb) were obtained from Ortho Pharmaceutical Co. (Ortho Japan, Tokyo), Leu1, Leu2a, Leu3a, Leu4 and HLA-DR MoAb were from Becton-Dickenson (Fujisawa Pharmaceutical Co., Tokyo). MoAb to LC (leucocyte common antigen) was purchased from Dako Immunoglobulins (Kyowa Medics Japan, Tokyo). Rabbit anti-mouse IgG $F(ab')_2$ fragments labelled with horseradish peroxidase (HRP) (Tago Inc., Maruzen Oil Chemical, Tokyo) was the second antibody.

Immunocytochemistry. Light microscopy. Cryostat sections to be observed by light microscopy were treated successively with 100% methanol and 0.03% hydrogen peroxide before being stained to inhibit the endogenous peroxidase activity (Streefkerk 1972). This was further inactivated by adding 10 mM sodium azide in Karnovsky's diaminobenzidine (DAB) solution.

In these sections, an indirect HRP-labelled antibody method was applied for the immunocytochemical staining using MoAb, as described (Shioda et al. 1984). This involves successive incubations with MoAb in optimal dilutions for 12 h and the 1:80 diluted HRP-labelled second antibody for 6 hours at 4° C. As negative controls, sections were incubated in PBS or ascitic fluid, which was obtained from mice injected with nonsecreting hybridoma cells, as the first



Figs. 1, 2. Immunohistochemical localization of OKM1− (Fig. 1) and OKM5− positive cells (Fig. 2a, b). OKM1 is reactive with mononuclear cells in the portal triad and sinusoids, whereas OKM5 is reactive with the sinusoidal lining cells. Central veins (*CV*), portal veins (*PV*) and arterioles (*A*) are unstained. (×150)

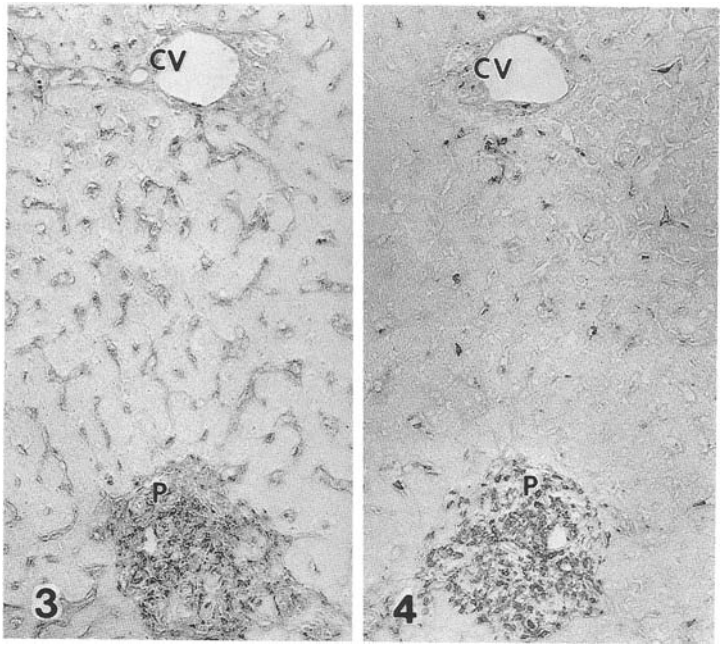
step. The sections were then incubated with Karnovsky's DAB solution (25 mg% diaminobenzidine tetrahydrochloride, Wako Pure Chemical Industries, Tokyo, and 10 mM hydrogen peroxide in 0.05 M Tris-HCl buffer, pH 7.6) containing 10 mM sodium azide, for 10 min, and counterstained with 1% methyl green solution, pH 4.0.

Electron microscopy. Cryostat sections adjacent to those taken for light microscopy were treated similarly through the antibody incubation steps; the sections were post-fixed in 1% glutaraldehyde in PBS. The step for the inactivation of endogenous peroxidase was omitted because at the ultrastructural level endogenous peroxidase-containing granulocytes can be easily differentiated, and endogenous peroxidase in KuC was eliminated by the post-fixation in glutaraldehyde in this staining procedure. The post-fixed sections were washed and incubated sequentially with 25 mg% DAB solution without hydrogen peroxide for 15 min and with 25 mg% DAB solution containing 10 mM hydrogen peroxide for 5 min. The sections were washed, reacted with 2% osmium tetroxide in PBS, dehydrated in graded ethanol, and embedded in Epon. Ultrathin sections, either unstained or stained with lead citrate, were viewed with a Hitachi H-300 electron microscope.

Results

The results of the immunohistochemical characterization of EnC in the liver are summarized in Table 1.

MoAb OKM1 reacted with only occasional mononuclear cells in the portal triads and sinusoids (Fig. 1). In contrast, MoAb OKM5 was reactive with sinusoidal lining cells but was unreactive with EnC of central veins, portal veins and arterioles (Fig. 2a, b). Electron microscopy confirmed that the reaction product of OKM5 was present along the external surface of the plasma membrane of the sinusoidal EnC and in endocytic-like invagina-



Figs. 3, 4. Immunohistochemical localization of Leu3a- (Fig. 3) and Leu1-positive cells (Fig. 4). The sinusoidal lining cells are reactive with Leu3a, whereas Leu1 does not reveal any reactivity with them. Lymphocytes in the portal triads (*P*) and sinusoidal spaces are stained. Central veins (*CV*) are unstained. (× 150)

Table 1. Immunohistochemical characterization of endothelial cells in the liver

Endothelial cells	Markers							
	OKM1	OKM5	HLA-DR	Leu1	Leu2a/OKT8	Leu3a/OKT4	Leu4	LC
Sinusoid	−	+	+	−	−	+	−	−
Portal vein	−	−	+	−	−	−	−	−
Portal artery	−	−	+	−	−	−	−	−
Central vein	−	−	+	−	−	−	−	−

+ : positive staining, − : negative staining

tion of the membrane (Fig. 8). Neither KuC nor Ito cells were reactive with OKM5.

The sinusoidal EnC were also strongly reactive with Leu3a (Figs. 3, 9) and OKT4 MoAb, but other T cell antigens, including OKT8, Leu1, Leu2a and Leu4, did not reveal any reactivity with the sinusoidal lining cells (Fig. 4).

HLA-DR was expressed by the majority of sinusoidal, venular and arteriolar EnC, and was also present on the mononuclear cells and KuC. However, Ito cells were unreactive (Figs. 5, 10). In addition, KuC and several

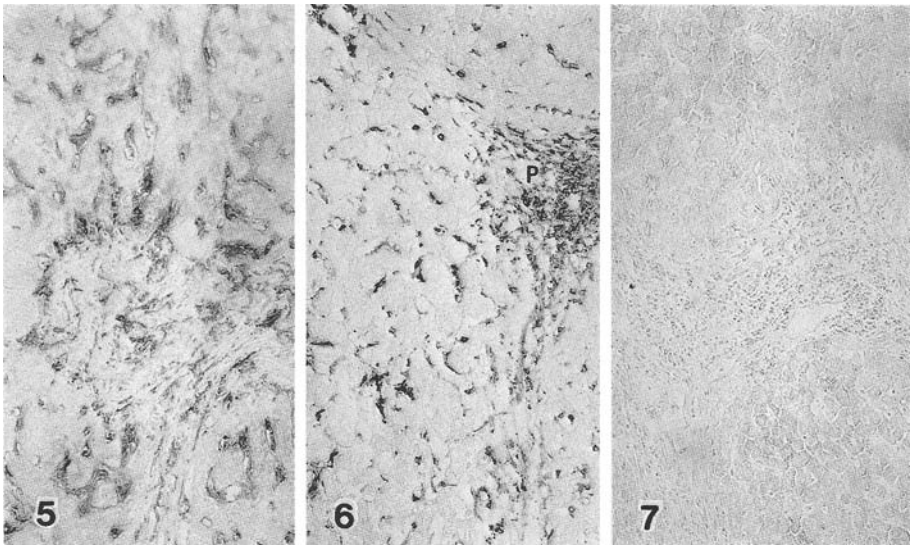


Fig. 5. Immunohistochemical localization of the cells expressing HLA-DR. The majority of sinusoidal endothelial cells are positive for HLA-DR. HLA-DR-positive Kupffer cells are also observed. (× 150)

Fig. 6. Immunohistochemical localization of LC-positive cells. Kupffer cells and several mononuclear cells in the portal triad (*P*) are positively stained. (× 150)

Fig. 7. Control staining using non-immune mouse protein instead of primary antibody. No reaction products are observed. (× 150)

mononuclear cells contained LC, but EnC and Ito cells were free from this antigen (Fig. 6). This was confirmed by immunoelectron-microscopic examinations (Fig. 11).

The control sections were uniformly negative (Figs. 7, 12).

Discussion

The important implications of the present work are that hepatic EnC heterogenously express surface antigens and that only the sinusoidal EnC share antigens with a peripheral blood macrophage subset which has been shown to be capable of presenting soluble antigens and inducing AMLR (Breard et al. 1980; Shen et al. 1983).

The induction of most immune responses requires the close cooperation of T cells and APC, such as cells of monocyte/macrophage lineage (Unanue 1978). Monoclonal antibodies OKM1 and OKM5 have been shown to detect antigenic determinants distributed on human peripheral blood APC subsets, and the OKM1 − OKM5 + subpopulation is capable of triggering AMLR (Shen et al. 1983). In addition, it has been reported that the stimulating capacity of peripheral blood mononuclear cells in AMLR is restricted to Ia-bearing cells, and that a MoAb specific for the β chain of HLA-DR

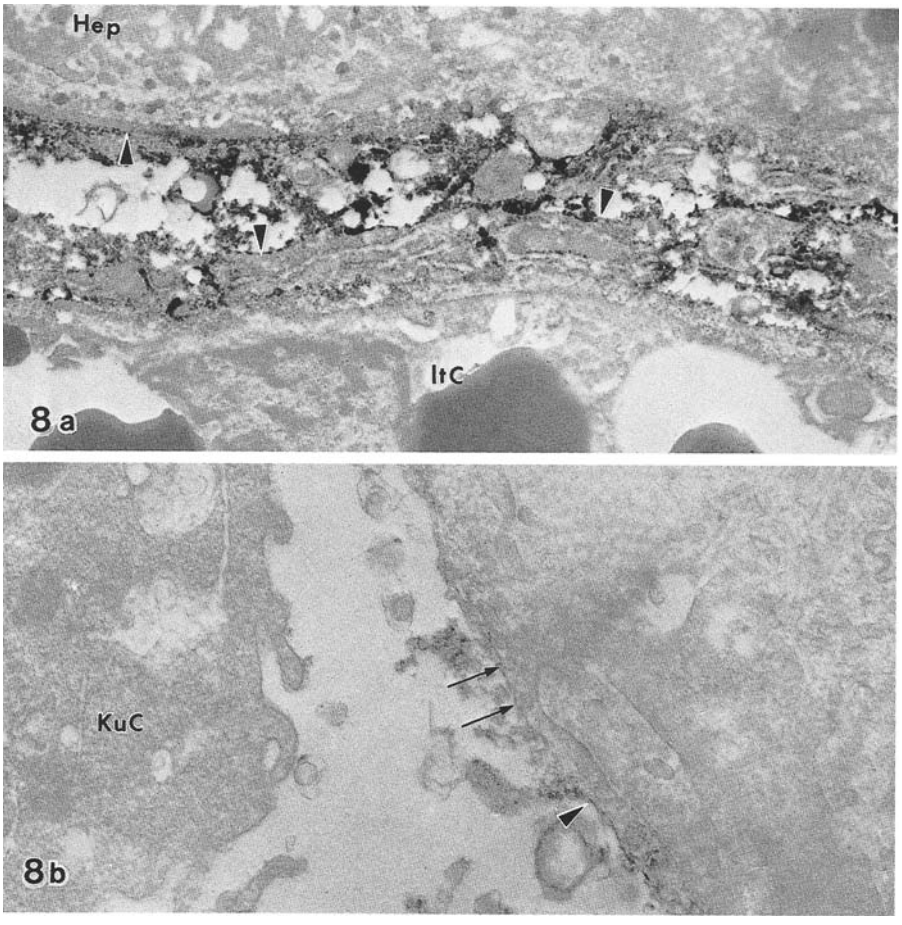


Fig. 8a, b. Ultrastructural localization of OKM5. **a, b** It is confirmed that Kupffer cells (*KuC*), Ito cells (*ItC*) and hepatocytes (*HeP*) are unreactive with OKM5, and the reaction products of OKM5 are present along the plasma membrane of the sinusoidal endothelial cells (◀) and in the endocytic invaginations (←). (**a** ×13,000, **b** ×15,000)

antigens inhibits AMLR (Mingari and Moretta 1982; Kasahara et al. 1984). However, previous investigators have shown that EnC express HLA-DR antigens and are capable of replacing macrophages in soluble antigen-induced T cell proliferation and of stimulating mixed lymphocyte reactions (Hirschberg et al. 1975; Moraes and Stastny 1977; Hirschberg et al. 1980; Nunez et al. 1983). More recently, it has been reported that human splenic sinusoidal lining cells also express the antigens found on helper/inducer (OKT4, Leu3a) and suppressor/cytotoxic (OKT8, Leu2a) T lymphocyte subsets, and antigens on a monocyte subset (OKM5) (Buckley et al. 1985). Thus, EnC may be important effector cells for immune responses and be involved in the induction and regulation of the immune responses.

In the past few years, it has been clarified that the liver and biliary

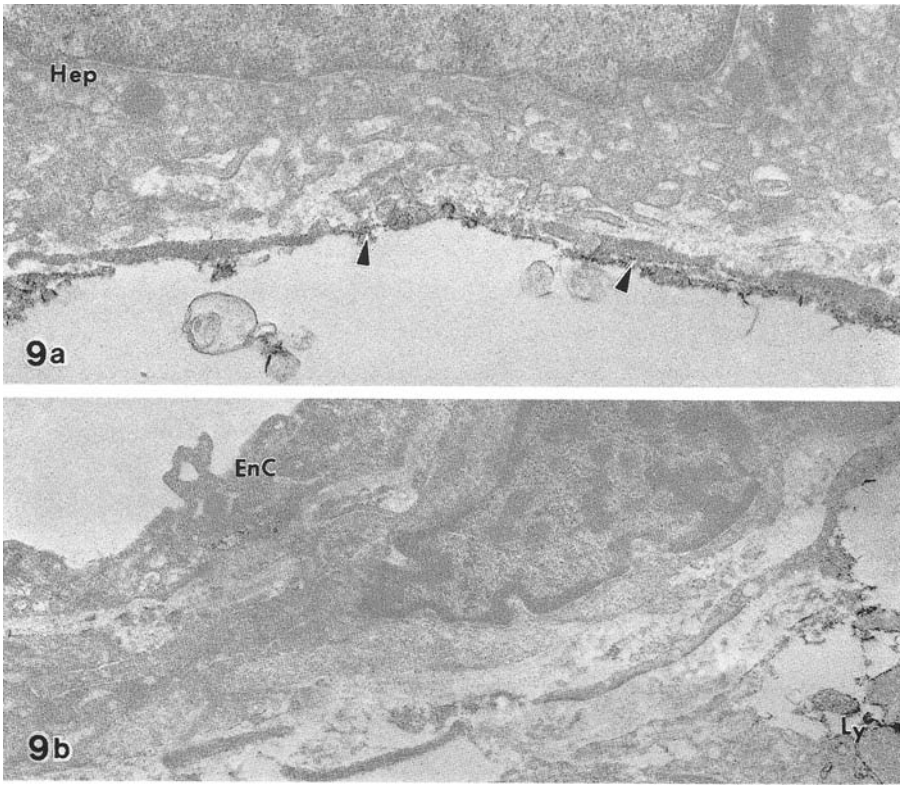


Fig. 9a, b. Ultrastructural localization of Leu3a. **a** The external surface of the sinusoidal endothelial cells (◀) is positively stained with Leu3a, **b** but the endothelial cells (EnC) in the portal triad are unreactive. The cytoplasmic projections of a Leu3a-positive lymphocyte (*Ly*) are observed. **a** ×8,000, **b** ×11,000)

system act as an integral part of immune responses, particularly of the mucosal immune system, in which secretory IgA plays an important role (Nagura et al. 1981; Kleinman et al. 1982). In recent years hepatic sinusoidal cells, which consist mainly of EnC and KuC, have received increasing attention with respect to their possible roles in immune responses or regulations. The sinusoidal EnC of the liver also demonstrate membrane staining for HLA-DR, OKM5, Leu3a and OKT4, but not for OKM1. EnC in the portal area and central veins, however, were HLA-DR+, OKM1−, OKM5− and Leu3a−. This suggests that the hepatic EnC heterogenously express surface antigens and that EnC of different anatomical compartments in the liver may play different immunological roles or functions. The results presented here also suggest that the sinusoidal EnC may substitute macrophages in immune responses.

In addition, the sinusoidal cells are well known to be responsible for the removal of various circulating antigens and immune complexes (Brown et al. 1982; Chandy et al. 1983), and both EnC and KuC are involved in

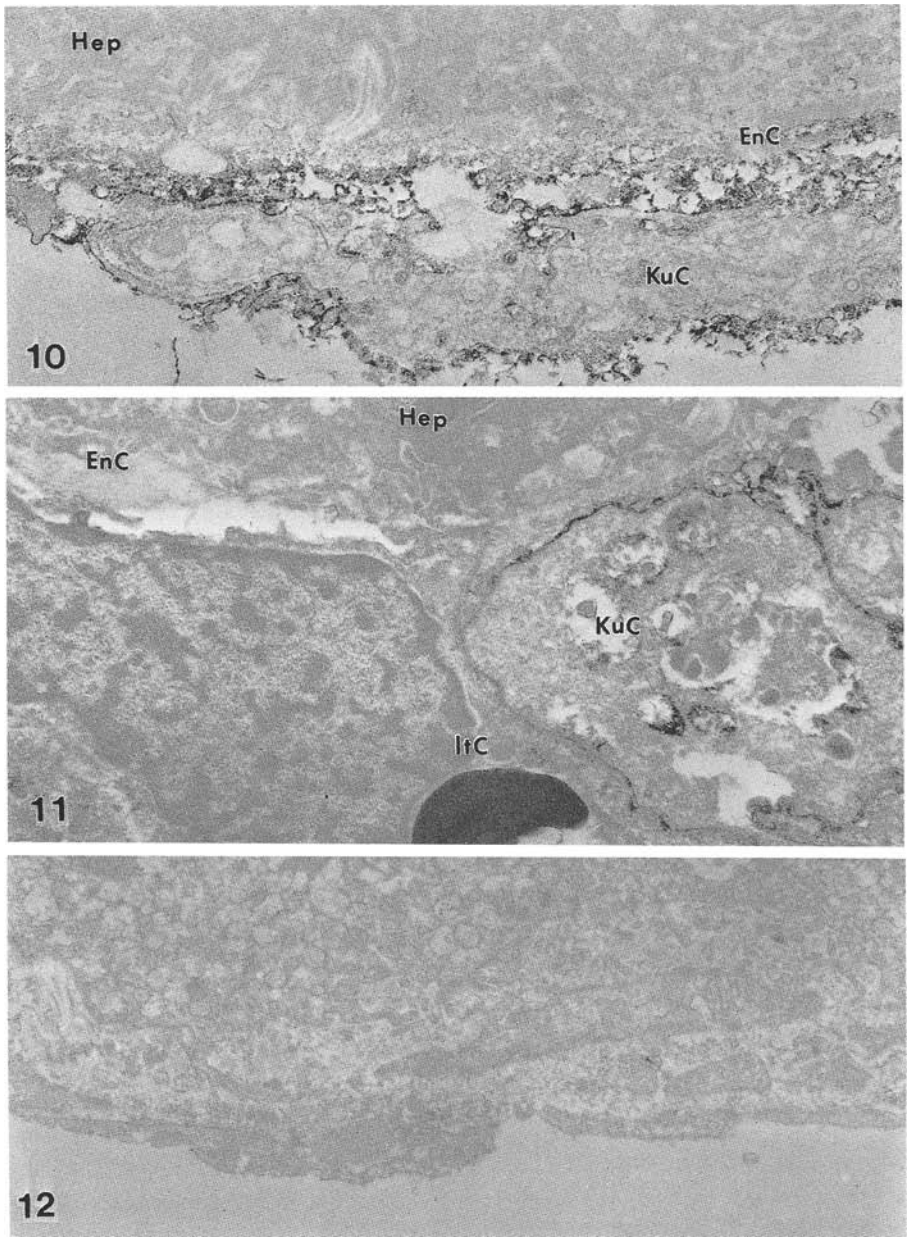


Fig. 10. Ultractructural localization of HLA-DR in the hepatic sinusoid. HLA-DR is expressed on the surface of the sinusoidal endothelial cells (EnC) and Kupffer cells (*KuC*), but not on the hepatocyte (*Hep*). (× 13,000)

Fig. 11. Ultrastructural localization of LC in the hepatic sinusoid. Only a Kupffer cell (KuC) expresses LC on the surface of the plasma membrane. *EnC*, endothelial cell; *ItC*, cell; *Hep*, hepatocyte. (× 13,000)

Fig. 12. Control staining using non-immune mouse protein is unreactive. (× 9,000)

this process (Praaning-van Dalen et al. 1981). KuC are known to constitute a group of fixed macrophages and to be responsible for the phagocytosis of most circulating antibodies and immune complexes. KuC were characterized by the expression of HLA-DR and LC antigens, which might be potentially considered as markers for phagocytic differentiation in the liver, similar to the mesangial phagocytes in the kidney (Schreiner and Unanue 1984). LC, which is probably homologous to the leucocyte-common antigen in the rat (Dalchau et al. 1980) and a major glycoprotein, an acceptor of sialic acid, unique to marrow-derived leucocytes or phagocytes (Standring and Williams 1978), is present only in KuC and several lymphoid cells in the portal area, but not in EnC. However, KuC failed to react with monoclonal antibodies OKM5 and Leu3a.

It remains to be determined whether similar phenomena will be demonstrated in isolated sinusoidal EnC or KuC in culture. In addition, although MoAb have advantages in their homogeneity, defined specificity and constant affinity, some of their specificities may be cross-reactive with antigenic sites present of unrelated molecules because the MoAb detect only one determinant on a macromolecule (Shioda et al. 1984). Cross-reactivity also remains an important problem in the present immunohistochemical study.

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Accepted March 24, 1986