American Journal of Pathology, Vol. 143, No. 1, July 1993

Defect of Fc Receptors and Phenotypical Changes in Sinusoidal Endothelial Cells in Human Liver Cirrhosis

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To analyze the pathological changes occurring in Fc receptors (FcRs) in sinusoidal endothelial cells (SECs) in chronic liver diseases, we first characterized immunohistochemically the SEC FcRs by using monoclonal antibodies (MAbs) to FcRs and then investigated the distribution of the SEC FcRs by using peroxidase-antiperoxidase IgG complexes as a ligand on frozen sections. MAb 2E1 to FcRII reacted with SECs in a similar manner to peroxidase-antiperoxidase IgG and blocked the peroxidase-antiperoxidase IgG binding to SECs, whereas MAbs 3G8 and Leu-11b to FcRIII did not. FcRs in normal liver were found along the sinusoidal walls, except for those in the outer periportal zones, but FcRs in chronic active hepatitis and cirrhosis were intermittently or focally absent. The lengths of the FcR-positive portion of sinusoids in unit areas were respectively about 54% and 76% of the normal values in active and inactive cirrhosis. Where FcRs were absent, the MAbs CD36, CD31, and EN4 revealed the presence of sinusoids and, in active cirrhosis, frequently the thickening of liver cell plates. The FcR-negative SECs in the outer periportal zones of normal livers were different from the SECs of other sites in the presence of PAL-E antigen and a rich amount of EN4 antigen, though these sinusoids possessed Kupffer cells and no perisinusoidal deposition of laminin. The FcR-negative SECs in liver diseases occasionally presented the character of ordinary blood vessels, viz., PAL-E antigen, CD34 antigen, and a deficiency of Kupffer cells, regardless of perisinusoidal laminin deposition. However, they preserved the character of normally FcR-possessing SECs, viz., CD36 antigen, and a small amount of EN4 and CD31 antigens. These findings indicate that the outer-periportal SECs in normal livers are phenotypically different from other SECs and that the SECs in diseased livers frequently undergo phenotypical changes, including loss of FcRs, regardless of perisinusoidal laminin deposition, i.e., capillarization of the sinusoids. These phenotypical changes in SECs may reduce the capacity of FcR-mediated IgG-IC metabolism in diseased livers. (Am J Pathol 1993, 143:105–120)

Considerable attention has been paid to the liver sinusoidal endothelial cells (SECs) as the site of uptake of various soluble macromolecules, such as soluble IgG-immune complexes (IgG-IC),[1–4] mannose-terminated glycoprotein,[5,6] and waste extracellular matrix components including hyaluronic acid.[6,7] The SECs are thought to be specialized in receptor-mediated endocytosis of these macromolecules,[5–8] to ingest these macromolecules in fairly large quantities,[3–7] and to contain a number of the lysosomal enzymes with high activities.[9] For example, SECs possess Fc receptors (FcRs)[2,8,10,11] that specifically bind the Fc portion of immunoglobulin G (IgG) and endocytose and degrade IgG-IC[3] as Kupffer cells do. In human diseases, the increase in the level of circulating macromolecules taken up by SECs[6,12–14] and the transformation of sinusoids into capillaries[15–18] or other phenotypic changes in SECs[19,20] are frequently observed. Hence, an interesting question arises as to whether or not the receptor-mediated endocytoses of macromolecules in SECs are affected by liver diseases.

We have recently reported that CCl_4-induced rat liver cirrhosis,[21] which has been demonstrated in a delayed clearance of circulating immune complex-

Accepted for publication January 31, 1993.

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es,[22] shows a weakened reactivity of sinusoidal FcRs with their focal absence and, further, that the FcR-absent SECs ingest no IgG-IC.[21] We postulated that an abnormal appearance of circulating immune complexes associated with chronic liver diseases[12,13] may, at least to some extent, be attributed to the defect in sinusoidal FcRs, though the state of sinusoidal FcRs in human chronic liver diseases has not yet been analyzed. Therefore, it would be of particular interest to ascertain the properties of sinusoidal FcRs in human liver diseases.

In the present study, we thus investigated the localization of sinusoidal FcRs by using peroxidase-antiperoxidase IgG complexes (PAPIgG) as a ligand on frozen sections and found a focal deficiency in endothelial FcRs in human liver cirrhosis. Furthermore, to clarify the nature of the FcRs detected by PAPIgG and FcR-lacking SECs, we immunohistochemically analyzed FcRs, SECs, Kupffer cells, and laminin as a major component of the basement membrane.

Materials and Methods

Liver Specimens

Liver specimens from 47 patients with liver cirrhosis and/or chronic hepatitis were studied (35 with hepatocellular carcinoma and 12 without carcinoma; 29 men and 18 women), excluding patients with biliary cirrhosis, cardiac cirrhosis, and acknowledged alcohol abuse. Upon hepatectomy, transection of esophageal varices or other surgical procedures performed on patients, liver specimens were obtained by wedge biopsy immediately following the celiotomy incision to ensure against tissue degeneration during the surgical operation. Thirteen patients with early gastric carcinoma, from whom liver specimens were obtained in a similar manner as the patients with liver diseases, served as normal liver cases. Hepatic or other inflammatory diseases were excluded as far as possible from the normal livers. Informed consent was obtained from these patients before the operation. The study was approved by the Ethics Committee of Hamamatsu University School of Medicine. One part of each liver specimen was processed routinely for histological examination, and the other part was frozen in dry ice-acetone and stored at −80 C.

Tissue Preparation

Five-μ cryostat sections of the frozen liver tissues were fixed with isopropyl alcohol for 10 seconds, treated with acetone for 10 seconds to remove fat, and then washed in phosphate-buffered saline. Tissue sections for monoclonal antibody (MAb) My10 staining only were dried under a stream of air for 1 minute at room temperature and then fixed with 2% paraformaldehyde in 0.02 mol/L cacodylate buffer, pH 7.4, containing 70% ethanol, for 10 minutes at −20 C.

Detection of Fc Receptors

The method utilized to detect the FcRs using PAPIgG and its specificity have already been described in the previous paper.[8] In brief, the fixed-frozen sections were first incubated with PAPIgG for 30 minutes, then immersed in 20 mg/dl of 3,3′-diaminobenzidine-4 HCl, 0.05 mol/L Tris-HCl buffer, pH 7.6, containing 0.005% H_2O_2, 10^{-2} mol/L NaN_3, and 0.15 mol/L NaCl (DAB-H_2O_2) for 5 minutes. PAPIgG was prepared as described previously.[8] The concentration of horseradish peroxidase (HRP) in the PAPIgG solution was adjusted to 15 μg/ml spectrophotometrically at 403 nm (0.001 OD403 corresponded to 0.444 μg of lyophilized HRP protein).

Immunohistochemistry

Endothelial cells, Kupffer cells, laminin (LM), Fcγ-receptor II (FcRII) and Fcγ receptor III (FcRIII) were stained by indirect HRP-labeled antibody method. In brief, the fixed-frozen sections were incubated with the primary antibodies listed in Table 1 for 30 minutes, washed with phosphate-buffered saline, incubated with HRP-labeled secondary antibody for 30 minutes, and then immersed in DAB-H_2O_2 for 5 minutes at 15 C. For double staining of FcRs or some antigens stained by indirect HRP-labeled antibody method and other antigens, the other antigens were stained by indirect alkaline phosphatase (Alk-Pase)-labeled method. The sections incubated with a primary antibody and the Alk-Pase-labeled secondary antibody for 30 minutes each were treated for 5 minutes with 0.1 mol/L Tris-HCl buffer, pH 8.5, containing 1.0 mmol/L levamizole and 0.15 mol/L NaCl, and then incubated for 20 minutes at 37 C with 0.1 mol/L Tris-HCl buffer, pH 8.5, containing 0.01% naphthol AS-MX phosphate sodium salt, 0.06% fast blue BB diazonium sodium salt, 1.0 mmol/L levamizole, and 0.15 mol/L NaCl. Levamizole was used to eliminate the endogenous Alk-Pase activity.

Table 1. *Antibodies Used: Sources and Dilution for Immunostaining*

Antibodies	Source	Dilution	Ref. no.
Primary antibodies			
MAb PAL-E (anti-EnC)	SANBIO, Uden, Neth.	1:20	23
MAb EN4 (anti-EnC)	SANBIO, Uden, Neth.	1:20	24, 25
MAb 25F9 (antimacrophage)	Boeringer, Tokyo, Japan	1:20	26
MAb 2G6 (antilaminin)	Boeringer, Tokyo, Japan	1:20	27
MAb 3G8 (CD16)	IMMUNOTECH, Marseille, France	1:30	28
MAb Leu-11b (CD16)	Becton, Mountain View, CA	1:20	29
MAb JC/70 (CD31)	Dakopatts, Kyoto, Japan	1:20	30
MAb 2E1 (CDw32)	IMMUNOTECH, Marseille, France	1:30	31
MAb My10 (CD34)	Becton, Mountain View, CA	1:20	32
MAb OKM5 (CD36)	Ortho, Raritan, NJ	1:20	33
MAb EBM11 (CD68)	Dakopatts, Kyoto, Japan	1:20	34
Secondary antibodies			
HRP-anti-mouse Ig	Dakopatts, Kyoto, Japan	1:40	
Alk-Pase-anti-mouse Ig	Dakopatts, Kyoto, Japan	1:40	

Enc: endothelial cell.

Inhibition of PAPIgG Binding on SECs Using MAbs to FcRs

Before the staining for FcRs using PAPIgG, the tissue sections of several normal and diseased livers were pretreated with the MAbs to FcRs listed in Table 1 for 30 minutes at 15 C. Mouse IgG subtypes (IgG1: MOPC-21; IgG2a: UPC-10; IgG2b: MOPC-141; IgG3: J606B), IgA (MOPC-315), and IgM (TEPC-183)[35] were used as controls for MAbs for FcRs. Furthermore, we examined the competition of PAPIgG binding on SECs using MAb 2E1 (anti-FcRII, mouse IgG2a) and mouse IgG2a (UPC-10). To distinguish SECs from macrophages, some tissue sections were stained with MAb EBM11 (CD68), which reacts with macrophages and blood monocytes,[34] by indirect Alk-Pase-labeled method before the incubation of anti-FcR MAb solutions or PAPIgG solution containing competitors.

Morphometric Analysis

The areas of lobules or regenerative nodules and the lengths of FcR-positive sinusoids in the photomicrographs of the liver tissue sections were measured with an automatic image analyzer (Luzex III, Nihon Kogaku Co., Ltd., Tokyo, Japan). The average lengths of the FcR-positive portion of the sinusoids per 1.0 mm^2 in lobules or regenerative nodules were also determined. The data were subjected to statistical analysis by Student's *t*-test.

Results

Character of SEC FcRs Detected by Using PAPIgG

MAb 2E1 stained continuously sinusoidal walls, including EMB11 antigen-negative slender lining cells, i.e., SECs of normal (Figure 1A) and diseased livers, in a similar manner as PAPIgG (Figure 2A). The pretreatment with MAb 2E1 at a concentration of 2.5 to 10 μg/ml blocked PAPIgG staining on SECs incompletely and, at 20 μg/ml, blocked it completely (Figure 2, B and C). MAbs 3G8 and Leu-11b stained EBM11 antigen-positive cells, a few small round cells, and polymorphonuclear cells and revealed no continuous sinusoidal lining (Figure 1B). The pretreatment with MAbs 3G8 or Leu-11b at 20 μg/ml did not block the PAPIgG staining on SECs. Moreover, PAPIgG staining on tissue sections was not significantly influenced by the pretreatment with four mouse IgG subtypes, IgA, or IgM at the same concentration of MAb 2E1.

In the competition examinations, the addition of MAb 2E1 (IgG subclass: IgG2a) to the PAPIgG solution at a concentration of 2.5 to 10 μg/ml incompletely suppressed the PAPIgG staining on SECs and at 20 μg/ml completely suppressed it in a manner similar to the pretreatment with MAb 2E1 in Figure 2C. However, the addition of IgG2a (UPC10) at 20 to 40 μg/ml did not significantly influence the PAPIgG staining of SECs.

Normal Livers

Distribution of Fc Receptors and Kupffer Cells

The distributions of the FcRs in normal livers were identical to those previously reported.[8] In brief, brown reaction products from PAPIgG, representing FcRs, were recognized continuously along the sinusoidal walls, but were lacking in the outer periportal zones (Figure 3A and Table 2). The size of the FcR-lacking zone varied with each case.

MAb 25F9 stained the variable-shaped mononuclear cells that possessed basically cytoplasmic processes, i.e., Kupffer cells in sinusoidal walls, and

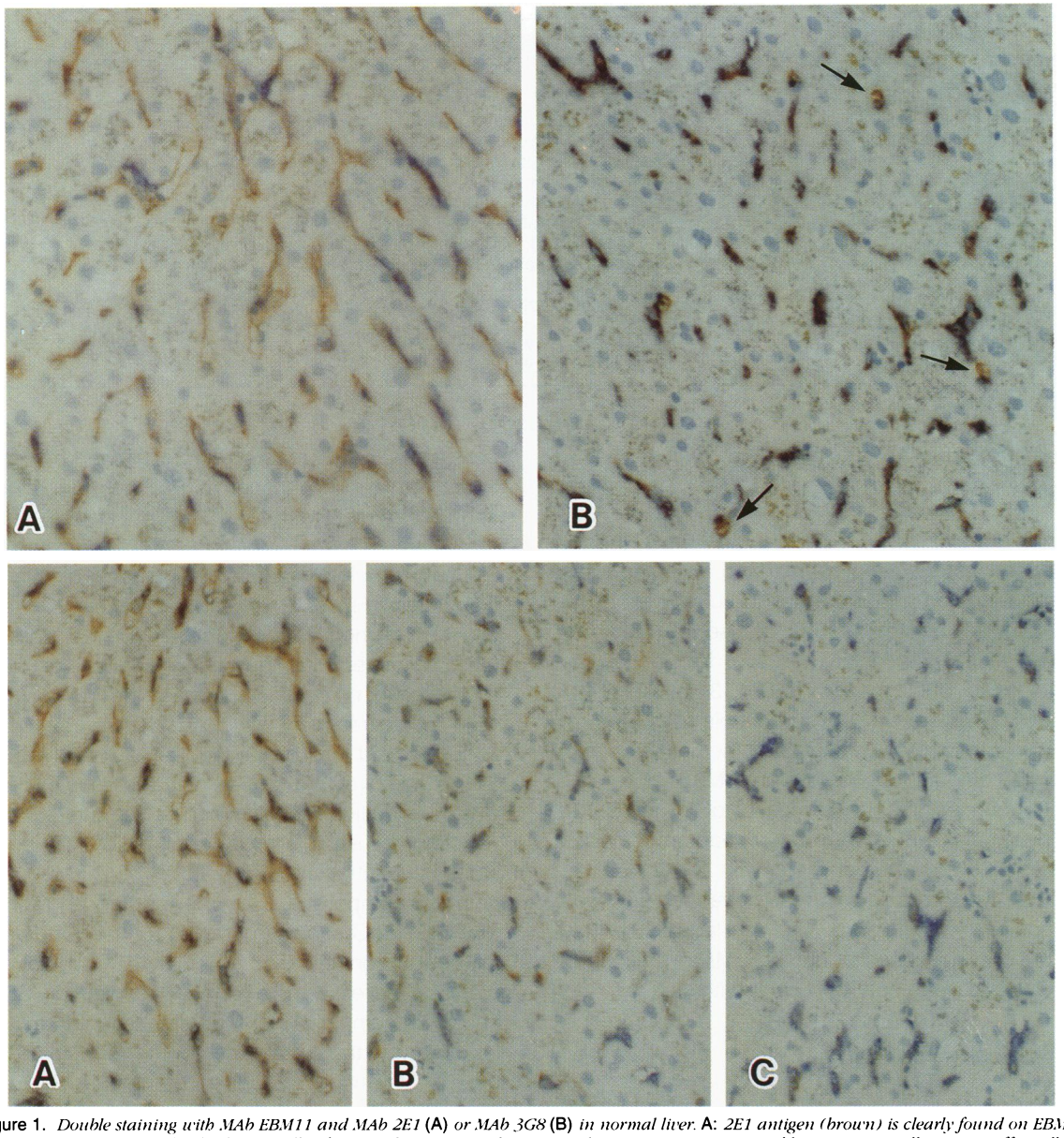

Figure 1. *Double staining with MAb EBM11 and MAb 2E1* **(A)** *or MAb 3G8* **(B)** *in normal liver.* **A:** *2E1 antigen (brown) is clearly found on EBM11 antigen (blue)-negative slender lining cells of sinusoids, i.e., SECs, but is not clear on EBM11 antigen (blue)-negative cells, i.e., Kupffer cells or blood monocytes.* **B:** *3G8 antigen (brown) is found on EBM11-positive cells and negative round cells (arrows), but not on SECs. Stained with hematoxylin (×200).*

Figure 2. *Suppression of PAPIgG staining by pretreatment with MAb 2E1.* **A:** *Control section of normal liver showing PAPIgG staining (brown) on SECs, i.e., EBM11 antigen (blue)-negative cells.* **B** *and* **C:** *Sections pretreated with MAb 2E1; suppression of PAPIgG staining on SECs is incomplete at the concentration of 5 μg/ml of specific protein* **(B)** *and complete at 20 μg/ml* **(C)**. *Stained with hematoxylin (×150).*

the large mononuclear cells in portal areas. Kupffer cells were solitarily scattered on the sinusoidal walls and were slightly more numerous in the central zone of the lobules (Figure 3B).

Staining for Endothelial Cells with Monoclonal Antibodies

MAb OKM5 clearly stained the endothelia of sinusoids including those in the FcR-negative areas of the outer periportal zones and a few small blood vessels (Figure 4A). MAb EN4 and MAb JC/70 stained the vascular and sinusoidal endothelia. The staining intensities of MAbs EN4 and JC/70 in sinusoids were far weaker than those in the other blood vessels, though MAb EN4 strongly stained the sinusoids in the outer periportal zones in a manner similar to the other blood vessels (Figure 4, B and C and Table 2).

MAb PAL-E clearly stained the endothelia of the capillaries and veins in the portal areas, sinusoids

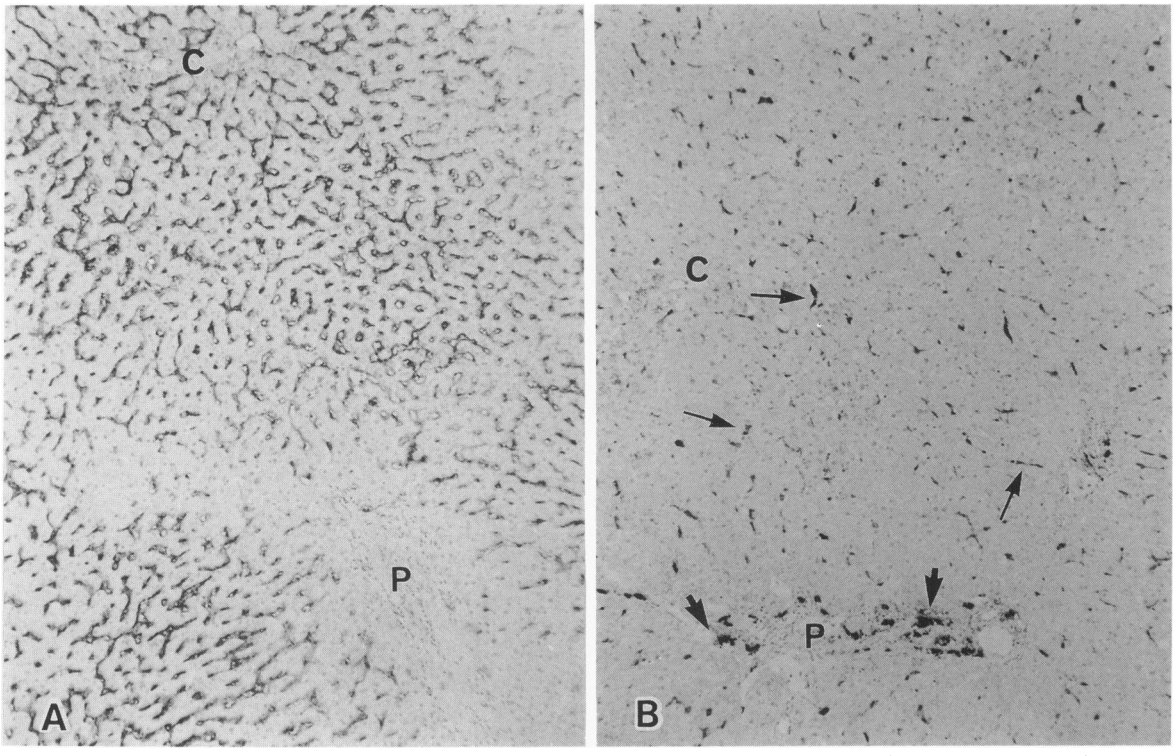

Figure 3. *Normal livers stained for FcRs and Kupffer cells.* **A:** *FcRs, represented by reaction products (black) from PAPIgG as a ligand, are recognized continuously along the sinusoidal walls, but are lacking in the outer periportal zones.* **B:** *Kupffer cells stained with MAb 25F9 (arrows) are scattered in the lobule. 25F9 strongly-positive large cells (arrow heads) in the portal areas are histiocytes. P: portal vein, C: central vein. Stained with hematoxylin (×80).*

Table 2. *Immunohistochemical Character of the Endothelia in Human Liver*

	FcR	Laminin	OKM5	EN4	JC/70	PAL-E	My10
Portal vein	–	++	–	++	+	++	+
Capillary	–	++	–>++	++	++	++	++
Hepatic artery	–	++	–	++	++	–	–
Central vein	–	+	–	+	+	+	–
Sinusoid in OPZ	–	–	++	+	+/–	++	–
Sinusoid (other)	++	–	++	+/–	+/–	–	–

–: no stain; +/–: weak staining; +: moderate staining; ++: marked staining; –>++: occasionally ++; OPZ, outer periportal zone.

in the outer periportal zones, and inlet venules that communicate with portal veins, but did not stain most of the sinusoids (Figure 5A). Upon double staining with MAbs PAL-E and 25F9, PAL-E-positive sinusoids occasionally accompanied 25F9-positive Kupffer cells (Figure 5A). MAb My10 stained the endothelia of the capillaries near the limiting plates and bile ducts, but no endothelia of sinusoids (Table 2).

Laminin

MAb 2G6 specific to LM[27] clearly stained the basement membranes of the central veins, blood vessels in portal areas, and bile ducts, but this MAb did not react with perisinusoidal spaces including those of FcR-negative areas in the outer periportal zones (Figure 5B). Upon double staining for LM and PAL-E antigen, PAL-E-positive sinusoids in the outer periportal areas were not stained with MAb 2G6 (Figure 5B and Table 2).

Chronic Hepatitis and Cirrhosis

Distribution of Fc Receptors and Kupffer Cells

The FcRs were recognized along the majority of sinusoidal walls. However, the sinusoidal FcRs in chronic active hepatitis and cirrhosis were intermittently or focally absent. The staining intensity for FcRs varied from case to case. Large mononuclear cells, infiltrated in the portal areas and fibrous septa, showed strongly-stained FcRs, though most

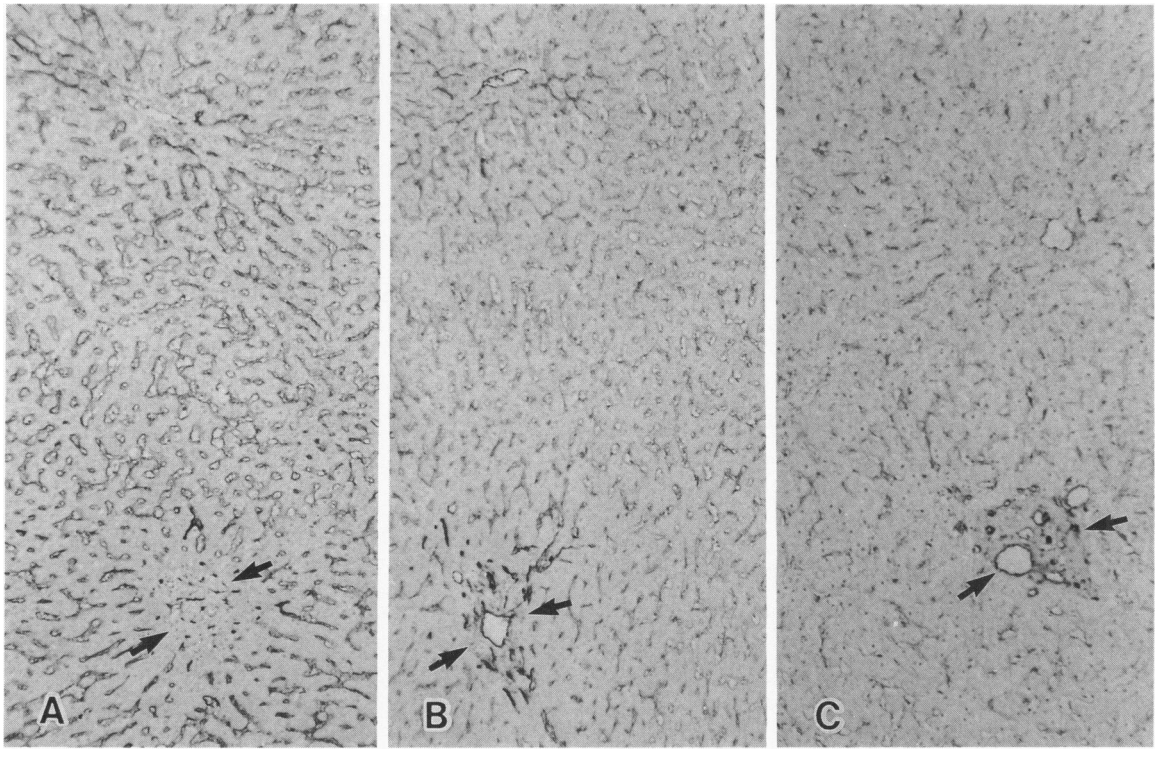

Figure 4. *Normal livers stained for endothelial cells by MAb OKM5* (A), *EN4* (B), *and JC/70* (C). *The sinusoids are clearly stained with MAb OKM5 and weakly with MAbs EN4 and JC/70, but those in the outer periportal zone are strongly stained with MAb EN4 in* B. *Arrowheads, portal areas. Stained with hematoxylin (×80).*

of the lymphoid cells did not. The quantitative differences among chronic hepatitis and cirrhosis are noted below.

In chronic active hepatitis without cirrhosis, sinusoidal FcRs were intermittently and focally absent in lobules, including the areas near the limiting plates surrounding the portal areas and fibrous septa (Figure 6A). 25F9-positive Kupffer cells were irregularly distributed with focal absence (Figure 6B). In chronic persistent hepatitis, sinusoidal FcRs were similar in distribution to normal liver, but the FcR-negative areas in the outer periportal zones were indistinct.

In liver cirrhosis, the absence of FcRs in cirrhosis with active chronic hepatitis, i.e., active cirrhosis, was seen in areas in regenerative nodules including the areas near the blurred limiting plates (Figure 7A) and was distinctly more marked than in inactive cirrhosis (Figure 8A). The 25F9-positive Kupffer cells were scattered in the regenerative nodules of cirrhotic livers, the density of Kupffer cells varied with the area, and variously-sized areas lacking in Kupffer cells were found irregularly in places in the regenerative nodules. This abnormal distribution of Kupffer cells was not pronounced in inactive cirrhosis (Figure 8B). Upon examination of adjacent serial sections, most of the FcR-absent areas were seen to correspond to the areas with few or no Kupffer cells (Figure 7B).

Staining for Endothelial Cells with Monoclonal Antibodies

MAbs OKM5, EN4, and JC/70 revealed the continuous networks of sinusoids in lobules and regenerative nodules. The adjacent serial sections or the sections doubly stained with PAPIgG and these antibodies showed the presence of sinusoids in the focally FcR-negative areas or in the intermittently FcR-negative portions of sinusoids. In the focally FcR-negative areas, thickened liver cell plates were frequently made apparent by these MAbs. The staining intensities of EN4 and JC/70 in most sinusoids, including those in FcR-negative areas, were weaker than in other blood vessels as seen in the FcR-positive sinusoids in normal livers (Figures 6C, 7C, 8C, 9, and Table 3).

MAb PAL-E clearly stained the endothelia of a varying number of sinusoids located near the limiting plates or in the interior of lobules and regenera-

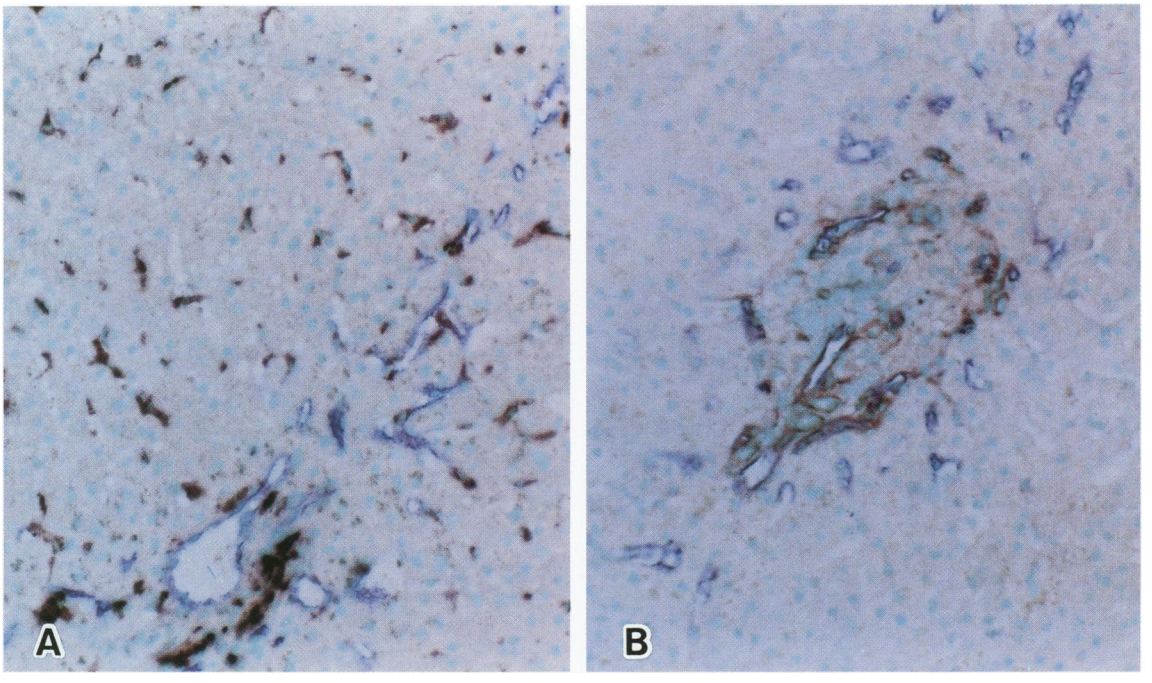

Figure 5. *Double stained normal liver with MAb PAL-E to endothelial cells and MAb 25F9 to Kupffer cells* **(A)** *or MAb 2G6 to laminin* **(B)**. **A:** *Kupffer cells (brown) are found in PAL-E antigen-positive sinusoidal walls (blue) in the outer periportal areas.* **B:** *Staining for laminin (brown) reveals the basement membranes of blood vessels and bile ducts in portal areas. Laminin is absent in sinusoidal walls, including those of PAL-E antigen-positive sinusoids (blue). Stained with methyl green (×150).*

tive nodules. However, most sinusoidal endothelia were not stained with MAb PAL-E. In the adjacent serial sections or on double staining for FcRs and PAL-E antigen, PAL-E antigen-positive sinusoidal endothelia were found in the FcR-negative areas, but the PAL-E-positive sinusoids represented only a portion of the FcR-negative sinusoids (Figures 6D, 7D, and 8D). MAb My10 occasionally stained sinusoids in the FcR-lacking areas. Furthermore, My10-positive sinusoids overlapped to some extent with the PAL-E-positive sinusoids on the double-stained sections (Figure 10 and Table 3).

Laminin

In chronic persistent hepatitis and inactive cirrhosis, only a few LM-positive sinusoids were found in the parenchyma, including the areas in the vicinity of the limiting plates. In chronic active hepatitis and active cirrhosis, LM fragmentarily or continuously outlined sinusoids in varying staining intensities and varying frequencies in lobules and regenerative nodules, including the areas in the vicinity of the blurred limiting plates. In general, the perisinusoidal deposition of LM was pronounced in active cirrhosis. In the adjacent serial sections, LM-positive sinusoids were found in the FcR-negative areas, but only a part of the FcR-negative sinusoids showed the perisinusoidal deposition of LM (Figures 6E, 7E, 8E, and Table 3). Moreover, in the serial sections, PAL-E antigen was frequently positive in the LM-positive sinusoids, but LM-positive sinusoids represented only a portion of the PAL-E-positive sinusoids (Figures 6D, 7D, and 8D).

Total Length of FcR-Bearing Sinusoids

The lengths of FcR-positive sinusoids in chronic active hepatitis, inactive cirrhosis, and active cirrhosis were significantly shorter than those in normal liver and chronic persistent hepatitis. The lengths of the FcR-positive portion of sinusoids in unit areas were 54% and 76% respectively of the normal values in active and inactive cirrhosis. The presence of circulating HBsAg or HBcAg did not significantly influence the distribution of sinusoidal FcRs (Figure 11).

Discussion

The presence of FcRs, which specifically bind the Fc portion of IgG, on SECs was suggested by using

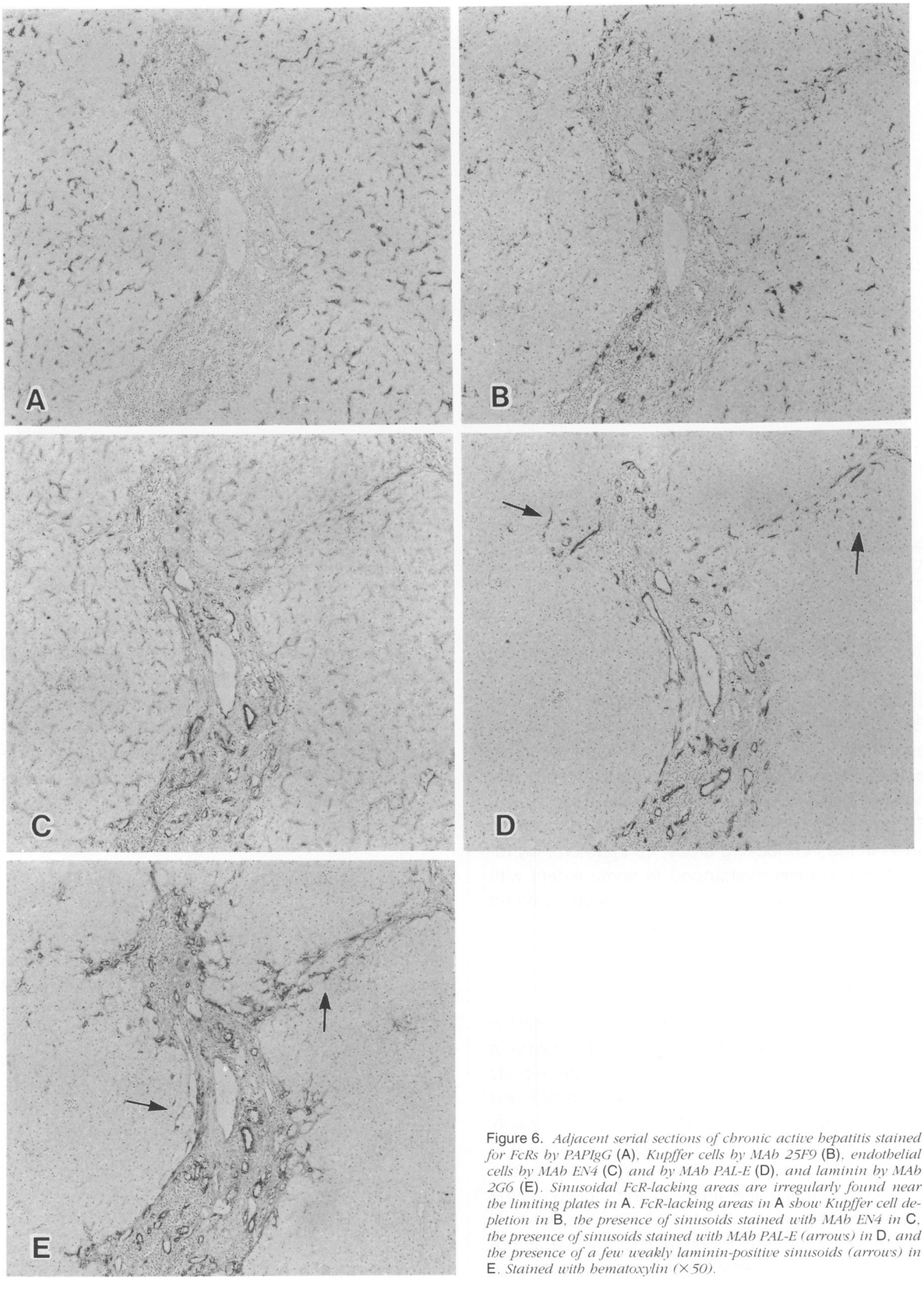

Figure 6. *Adjacent serial sections of chronic active hepatitis stained for FcRs by PAPIgG* (A), *Kupffer cells by MAb 25F9* (B), *endothelial cells by MAb EN4* (C) *and by MAb PAL-E* (D), *and laminin by MAb 2G6* (E). *Sinusoidal FcR-lacking areas are irregularly found near the limiting plates in* A. *FcR-lacking areas in* A *show Kupffer cell depletion in* B, *the presence of sinusoids stained with MAb EN4 in* C, *the presence of sinusoids stained with MAb PAL-E (arrows) in* D, *and the presence of a few weakly laminin-positive sinusoids (arrows) in* E. *Stained with hematoxylin (×50).*

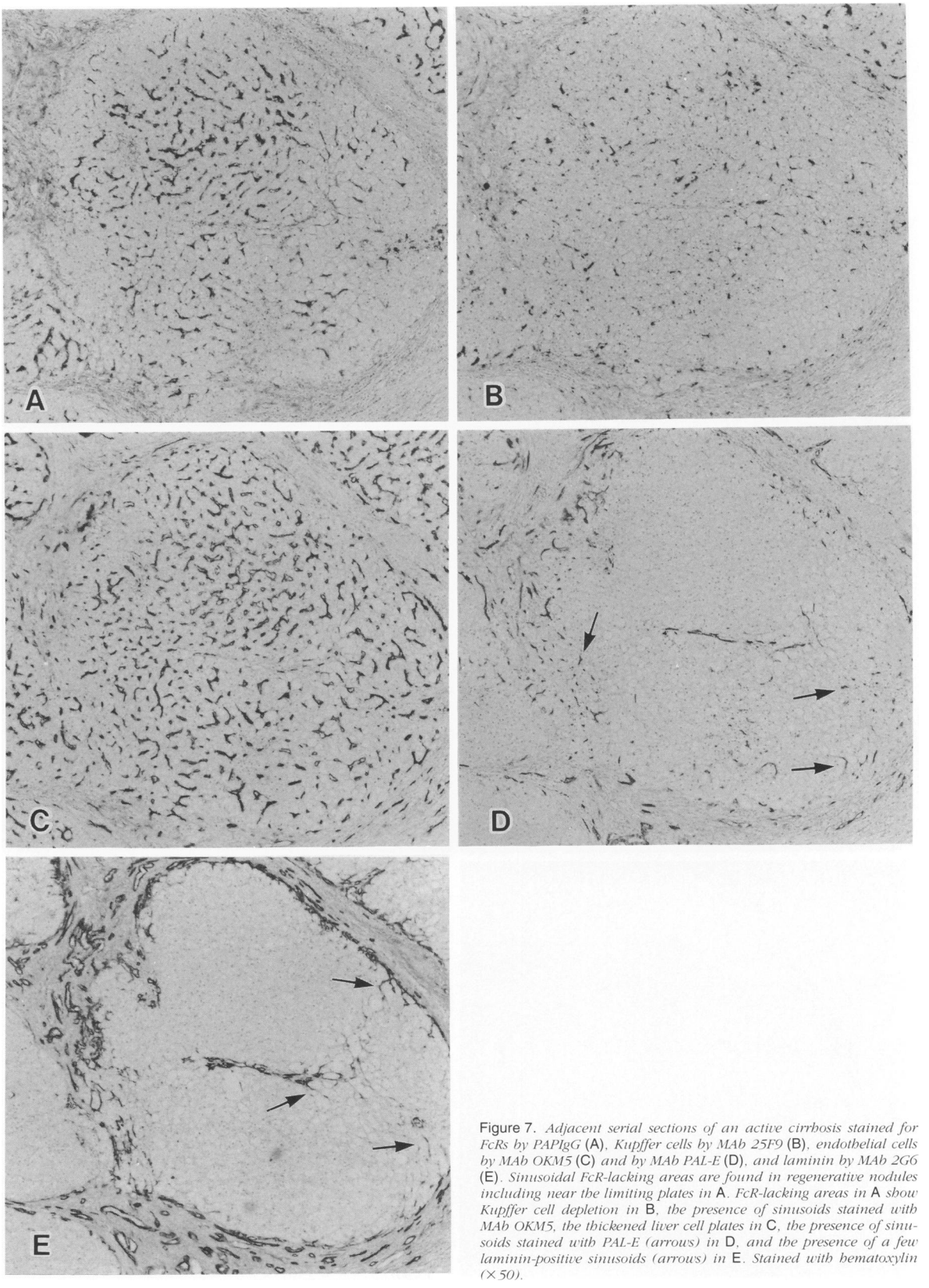

Figure 7. *Adjacent serial sections of an active cirrhosis stained for FcRs by PAPIgG* (A), *Kupffer cells by MAb 25F9* (B), *endothelial cells by MAb OKM5* (C) *and by MAb PAL-E* (D), *and laminin by MAb 2G6* (E). *Sinusoidal FcR-lacking areas are found in regenerative nodules including near the limiting plates in* A. *FcR-lacking areas in* A *show Kupffer cell depletion in* B, *the presence of sinusoids stained with MAb OKM5, the thickened liver cell plates in* C, *the presence of sinusoids stained with PAL-E (arrows) in* D, *and the presence of a few laminin-positive sinusoids (arrows) in* E. *Stained with hematoxylin (×50).*

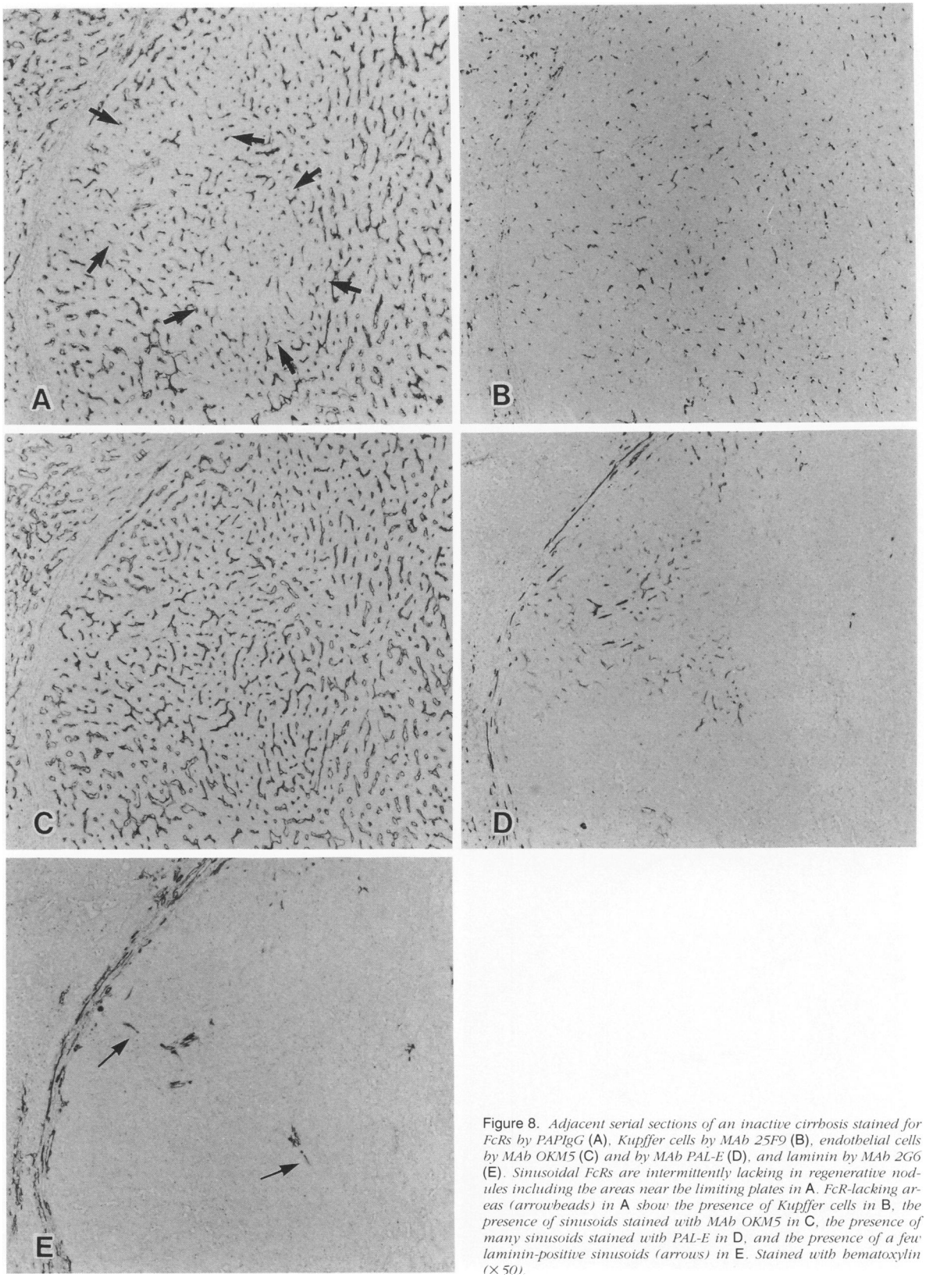

Figure 8. *Adjacent serial sections of an inactive cirrhosis stained for FcRs by PAPIgG* (A), *Kupffer cells by MAb 25F9* (B), *endothelial cells by MAb OKM5* (C) *and by MAb PAL-E* (D), *and laminin by MAb 2G6* (E). *Sinusoidal FcRs are intermittently lacking in regenerative nodules including the areas near the limiting plates in* A. *FcR-lacking areas (arrowheads) in* A *show the presence of Kupffer cells in* B, *the presence of sinusoids stained with MAb OKM5 in* C, *the presence of many sinusoids stained with PAL-E in* D, *and the presence of a few laminin-positive sinusoids (arrows) in* E. *Stained with hematoxylin (×50).*

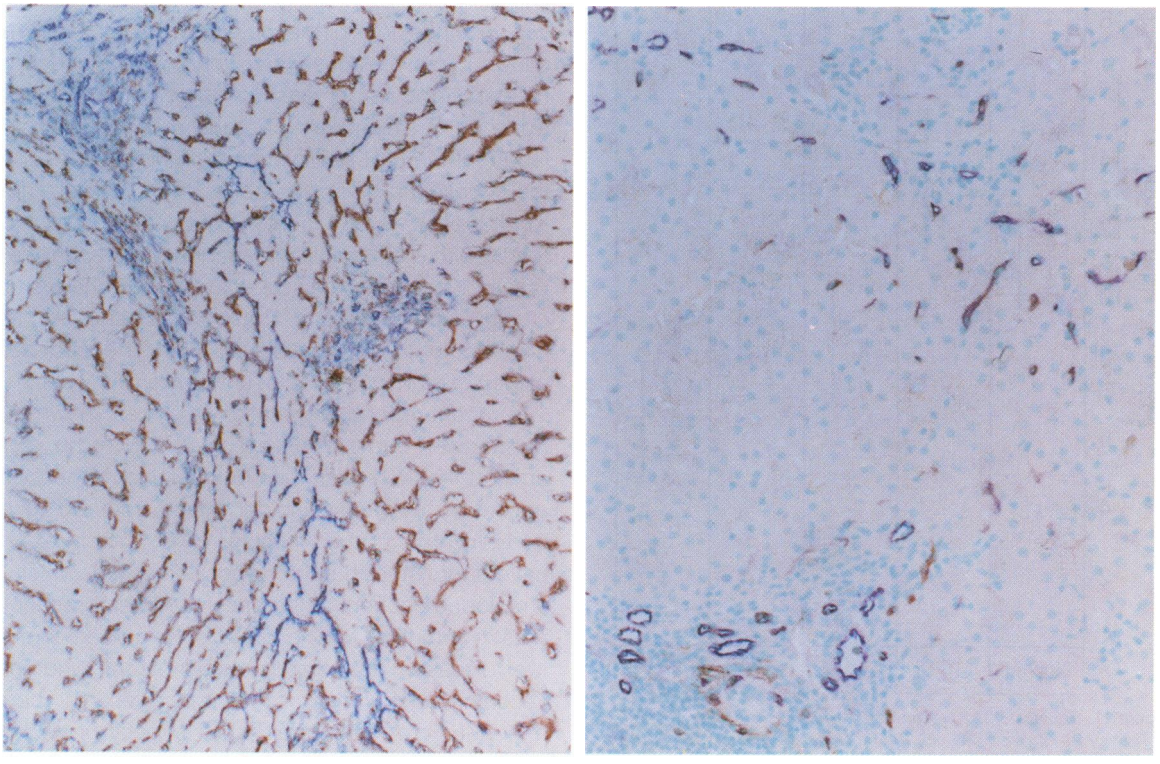

Figure 9. *Double staining for FcRs by PAPIgG and endothelial cells by MAb JC/70 in active cirrhosis. FcR (brown)-negative portions of sinusoids are intermittently or continuously stained with MAb JC/70 (blue). The blue staining of MAb JC/70 in most of the FcR-positive sinusoids is blocked by the brown reaction products of 3,3'-diaminobenzidine from FcRs. Stained with methyl green (×75).*
Figure 10. *Double staining for endothelial cells by MAb PAL-E and MAb My10 in an active cirrhosis. PAL-E antigen (blue) overlaps on one part of My10 antigen-positive sinusoids (brown) in varying staining intensities. Stained with methyl green (×120).*

Table 3. *Immunohistochemical Character of the FcR-Positive or Negative Sinusoidal Endothelia in Chronic Liver Diseases*

	Laminin	OKM5	EN4	JC/70	PAL-E	My10
FcR-positive sinusoids in						
CAH	−	+	+/−	+/−	−>>+	−
Inactive cirrhosis	−	+	+/−	+/−	−>>+	−
Active cirrhosis	−	+	+/−	+/−	−>>+	−
FcR-negative sinusoids in						
CAH	−>>>+*	+	+/−	+/−	−>>+	−>>>+
Inactive cirrhosis	−>>>+*	+	+/−	+/−	−>>+	−>>+
Active cirrhosis	−>>+*	+	+/−	+/−	−>+	−>>+

−: no stain; +/−: weak staining; +: clear staining; −>+, −>>+, and −>>>+: mean low, lower and lowest frequency of sinusoids showing +; +*: various staining. CAH, chronic active hepatitis.

IgG-sensitized erythrocytes or bovine serum albumin-anti-bovine serum albumin (BAB) IgG complexes on isolated cells and isolated perfused livers, though the specificity of receptors for the Fc portion of IgG was not elucidated.[2,10] Our study[8] using PAPIgG complexes and PAPF(ab)'$_2$ complexes without the Fc portion of IgG has indirectly demonstrated the presence of FcRs on SECs through the competition experiments with monomeric and heat-aggregated IgG thought to be important to the demonstration of FcRs and through the suppression experiments using anti-C1q and several carbohydrates to eliminate the participation of the C1q- or carbohydrate-mediated mechanism. In addition, we have proved that SECs ingest BABIgG complexes injected intravenously and degrade this IgG-IC in lysosome but do not ingest BAB F(ab)'$_2$ complexes without the Fc portion of IgG.[3,21]

FcRs to IgG are now classified into FcRI, FcRII, and FcRIII.[36] FcRI has a high affinity for ligand and is the only FcR for which the binding of monomeric IgG can be measured directly. Although the affinities of FcRII and FcRIII for ligand are so low that the binding of monomeric ligand is difficult to measure directly, the presence of these FcRs is readily demonstrated by the binding of immune complexes. Recent reports[37–39] have shown that anti-FcRII and

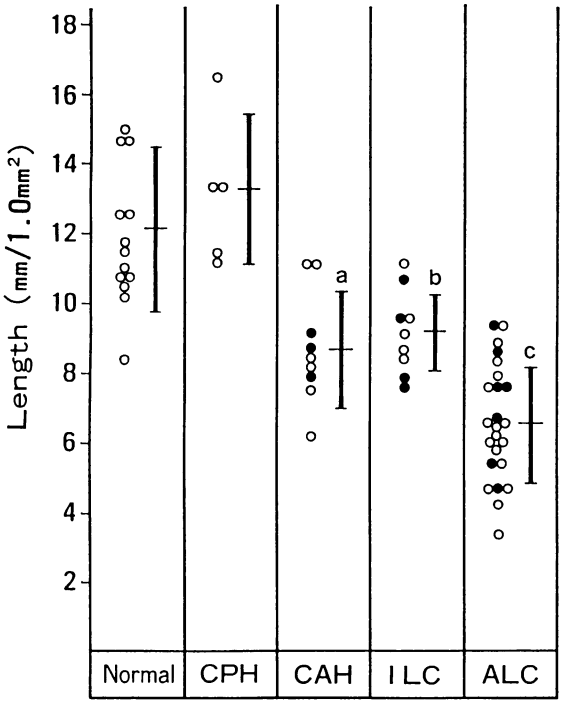

Figure 11. *Lengths of the FcR-positive portion of sinusoids in lobules or regenerative nodules. ●: circulating HBsAg and/or HBcAg-positive; ○: circulating HBsAg and/or HBcAg-negative; Normal: normal liver; CPH: chronic persistent hepatitis; CAH: chronic active hepatitis; ILC: inactive liver cirrhosis; ALC: active liver cirrhosis. Each bar represent the mean ± standard deviation (S.D.). a,b,c: significantly different from normal livers ($P < 0.01$); C: significantly different from chronic active hepatitis and inactive cirrhosis ($P < 0.01$).*

FcRIII MAbs, including MAbs 2E1, 3G8, and Leu-11b, immunostained sinusoidal endothelia based on the observation of a sinusoidal pattern of staining, although the endothelial nature of these stainings has not been definitively established.

In this study, by using a cell marker, i.e., MAb EBM11, for tissue macrophages and blood monocytes,[34] we demonstrated that FcRII antigen stained with MAb 2E1 was present in SECs, but that the presence of the FcRIII antigen in SECs could not be confirmed by using anti-FcRIII MAbs 3G8 and Leu-11b, and that the PAPIgG binding on SECs in frozen sections was suppressed by the pretreatment and competition with MAb 2E1 thought to block ligand binding.[36,40] From these results, the existence of FcRs, especially low-affinity FcRs in SECs, has become more certain. Although further study will be necessary, FcRII probably mediates the PAPIgG binding on SECs in frozen sections.

We used PAPIgG to stain FcRs in normal and chronically diseased human livers because PAPIgG reacts with FcRs as a ligand and is conveniently double stained with many MAbs. Although the sinusoidal FcRs in normal livers were recognized continuously along the sinusoidal walls with the exception of sinusoids in the outer periportal zones as shown in our previous study,[8] chronic active hepatitis and cirrhosis demonstrated intermittent or focal absence of sinusoidal FcRs and a decrease in the length of the FcR-positive sinusoids in unit areas. In the focally FcR-lacking areas, no or only a few Kupffer cells were found by using MAb 25F9, which reacts with a differentiation antigen preferentially expressed on mature tissue-fixed macrophages including Kupffer cells.[26] These results suggest that alteration of the character of SECs or the focal deficiency of sinusoids may occur in liver diseases.

A recent report on cirrhotic liver[16] has shown that Kupffer cell depletion is associated with the presence of the basement membrane, which is normally absent in the perisinusoidal space, referred to as capillarization of the sinusoids.[15–18] Bianchi et al[17] have suggested that the deposition of LM, which is a major component of the basement membrane, occurs in company with its formation in human chronic liver diseases, based on light microscopic and conventional electron microscopic observations. Thereafter, Martinez-Hernandez[18] has directly proved by using immunoelectron microscopy that newly deposited LM is found in the newly formed basement membrane in the rat CCl_4-induced cirrhosis, but not in normal perisinusoidal spaces. Also, in this study, LM deposition in the perisinusoidal spaces of human liver diseases were occasionally found by using anti-LM MAb 2G6,[27] which reacted with the basement membranes of bile ducts and blood vessels but not with perisinusoidal spaces of normal livers.

The LM distribution in the perisinusoidal spaces of normal adult livers is controversial. Under the conditions in which the basement membranes of bile ducts and blood vessels have been clearly stained with polyclonal antibodies for LM, some investigators have shown the presence of LM in the perisinusoidal spaces in normal livers,[14,41,42] whereas others have not.[17,18,21,43,44]

The discrepancies among studies on the recognition of perisinusoidal LM may be related not only to the difference in the tissue preparation as shown by Griffiths et al,[41] but also to the difference in the specificity or reactivity of polyclonal antibodies, which are thought to be a mixture of antibodies having various affinities for the many antigen sites of LM. LM has three subunits, B1, B2, and A, but the presence of subunit S, subunit M, and isoforms of LM lacking A or B1 is known in many organs.[45] A study using MAbs and peptide antisera to LM subunits by Wewer et al[44] has shown that LM subunits

B1, B2, S, and M, assumed to constitute two isoforms of LM, M-B1-B2 and M-S-B2, accumulate transitorily in the perisinusoidal spaces in neonatal and posthepatectomy rat livers and that subunit A is always found in basement membranes of blood vessels but in no perisinusoidal spaces in neonatal and adult rat livers. It is thus reasonable to regard the LM-negative sinusoids demonstrated by the use of MAb 2G6 in this study as possessing no basement membrane similar to that of capillaries, and the LM-positive sinusoids as possessing a newly formed basement membrane as described by Martinez-Hernandez[18] or as the transitory deposition of LM in the regenerative process.[43,44] In the adjacent serial sections, the perisinusoidal deposition of LM occupied only a part of the FcR-free areas. This finding suggests that the absence of endothelial FcRs and Kupffer cells is not a phenomenon that follows the formation of basement membrane, i.e., capillarization of sinusoids.

The existence of sinusoids in the FcR-lacking areas, including those of normal livers, was confirmed by staining SECs with MAbs OKM5, EN4, and JC/70. MAb OKM5, thought to be a CD36 antibody for thrombospondin receptors,[33] reacts with some vascular endothelial cells, including SECs and a few capillaries in portal areas.[20,46] MAb EN4, considered to be an antibody for all endothelial cells including those of lymphatic vessels,[24,25] weakly stained sinusoids, but strongly stained other vessels and the sinusoids in the outer periportal zone. MAb JC/70, believed to be a CD31 antibody that recognizes a membrane glycoprotein[30] and features the broadest endothelial positivity that is superimposable onto the EN4 pattern,[25] faintly stained all sinusoids. In the FcR-lacking areas of chronic active hepatitis and cirrhosis, these antibodies frequently revealed the thickening of liver cell plates, which is thought to represent the active or recent regeneration of liver cells.[43,47] Moreover, the depletion of the FcR activity of SECs has been found to occur during the recovery period of D-galactosamine-induced liver injury in rats.[4] Therefore, part of the FcR deficiency and other changes in SECs may have appeared in association with the destructive or regenerative process of hepatitis or liver cirrhosis.

The SECs in the outer periportal zones of normal livers may be phenotypically different from the SECs of other sites in the absence of FcRs, of a rich amount of EN4 antigen, and of an antigen stained with MAb PAL-E, which reacts with the endothelial cells of blood vessels except for large arteries,[23] though these sinusoids occasionally possess Kupffer cells and no LM-positive basement membrane similar to that of the capillary. Heterogeneity has been observed between periportal SECs and centrilobular SECs in normal rat liver, based on the number of fenestrae[48] or on analysis by sedimentation velocity.[49] In rat liver, however, the absence of SEC FcRs in the outer periportal zones has not been observed in our previous reports.[8] These immunohistochemically demonstrable differences in SECs may be specific in human or other species.

The FcR-negative SECs in chronic hepatitis and cirrhosis occasionally, if not always, possessed phenotypic characteristics of the ordinary blood vessels, i.e., the expression of PAL-E antigen and the CD34 antigen stained with MAb My10, which reacts with a 110-kd glycoprotein on hematopoietic progenitor cells and the endothelial cells of capillaries,[32] and a deficiency of Kupffer cells. However, they preserved the characteristics of normally FcR-possessing SECs, viz., OKM5 (CD36) antigen, and a small amount of EN4 and JC/70 (CD31) antigens. The well-known phenotypical change in SECs in chronic liver diseases is capillarization of sinusoids,[15–18] which means morphologically the appearance of newly formed basement membranes and the loss of the fenestrations of SECs,[15,18] and is histochemically accompanied by the perisinusoidal deposition of laminin, the expression of Ulex europaeus I lectin-binding sites on SECs,[19] and a large amount of factor VIII-related antigen on SECs.[14,20] The defect of SEC FcRs was distributed far more widely than the perisinusoidal LM deposition, i.e., capillarization of sinusoids, but involved the sinusoids featuring LM deposition.

The focal absence of sinusoidal FcRs with Kupffer cell depletion has also been found in CCl_4-induced rat liver cirrhosis,[21] but the sinusoids with LM deposition are not always devoid of FcRs in contrast to the sinusoids with LM deposition in human livers. These discrepancies among human and rat concerning the relationship between FcR deficiency and perisinusoidal LM deposition may relate to the difference in the pathogenesis or to the speed of the progress of liver injury. In either case, the phenotypical changes, including the absence of endothelial FcRs with Kupffer cell depletion, seems to be capable of occurring independently of the capillarization of sinusoids.

Recently, Martinez-Hernandez and Martinez[50] have shown that the capillarization of sinusoids in CCl_4-induced rat liver cirrhosis disturbs the bidirectional exchange of macromolecules normally taking place between hepatocytes and blood. However,

the SECs not only play an important role in the macromolecular exchange between hepatocytes and blood, but also the SEC itself is specialized in receptor-mediated endocytosis of various macromolecules, including soluble IgG-ICs.[3–7] In CCl_4-induced rat liver cirrhosis,[21] in which has been demonstrated a delayed clearance of immune complexes[22] and the focal absence of sinusoidal FcRs with Kupffer cell depletion, the IgG-IC and colloidal carbon injected intravenously have been ingested only by FcR-possessing SECs and Kupffer cells and not by FcR-lacking SECs.[21]

We can thus assume that the FcR-lacking SECs are not capable of ingesting IgG-ICs. The capacities of FcR-mediated metabolism in the sinusoids are probably reduced in the patients with liver cirrhosis, in whom the increased level of circulating immune complexes is occasionally found. Moreover, the results presented here suggest that the FcR-lacking SECs may be changed in other types of receptor-mediated endocytoses because the FcR-lacking SECs, accompanied by various phenotypic changes in human cirrhosis, have exhibited a loss in their capacity to ingest the colloidal carbon injected intravenously in rat cirrhosis.[21]

Acknowledgments

The technical assistance of Masaaki Kaneta and Akemi Yamamoto is gratefully acknowledged.

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