IMMUNOPATHOLOGICAL STUDIES OF ORTHOTOPIC HUMAN LIVER ALLOGRAFTS

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Summary

Twenty-six specimens obtained from twenty human orthotopic liver allografts 10–968 days after transplantation were studied by light microscopy, electron microscopy, and immunofluorescence. The main lesions consisted of mononuclear-cell infiltration around the portal tracts, centrilobular cholestasis, liver-cell atrophy and reticulin collapse, obliterative intimal thickening of hepatic arteries, and fibrosis. Moderate amounts of IgG and/or IgM and complement (β1C/β1A globulin or C'lq) were observed in four of the liver samples and smaller deposits were present in another five. A further three specimens contained IgG without complement. IgA was detected in only one of the samples. The immunoglobulins were found in the walls of the portal and central veins and of the sinusoids in all thirteen positive liver samples, in the walls of branches of the hepatic artery in three, and in the cytoplasm of some of the mononuclear cells infiltrating the portal tracts in nine of the specimens. Fibrinogen was seen in eight of the samples, usually in the spaces of Disse. Accumulations of immunoglobulins and complement were less frequent in liver than in kidney and heart allografts. These findings suggest that in the failure of human liver allografts cell-mediated immunity and non-immunological factors may be more important than humoral antibody.

Introduction

Morphological and immunopathological studies of human renal [1-9] and cardiac [10, 11] allografts have shown that circulating immunoglobulins and complement probably play an important part in the rejection of these organs. In this report we seek evidence of the same mechanism in hepatic allografts. Twenty-six specimens obtained from twenty orthotopic allogeneic liver grafts 10–968 days after transplantation were examined immunopathologically. The findings suggest that deposition of immunoglobulins and complement in human hepatic allografts is less frequent and less intense than in renal and cardiac allografts protected by similar immunosuppressive regimens.

Materials and Methods

Liver Specimens

Twenty-six liver specimens (table ı) obtained from twenty hepatic allografts were studied by light and electron microscopy and by immunofluorescent techniques. Fourteen of the transplants, indicated by the letters OT, were from the University of Colorado Medical Center, and six, indicated by the letters OL, were from Addenbrooke's Hospital, Cambridge, and King's College Hospital, London. The commonest indications for liver replacement were primary hepatic malignancy and biliary atresia. Fifteen of the specimens were obtained by aspiration needle or by open surgical biopsy, four at removal of the graft (and replacement with a fresh allograft in three of the cases), and seven at necropsy. All the patients received prednisone and azathioprine. Seventeen were also treated with horse antilymphocyte globulin (A.L.G.). In four patients this was for 5–10 days only. The number of days after transplantation when the specimen was taken, together with additional clinical data, are given in table ı. Morphologically normal liver tissue, obtained accidentally during percutaneous renal biopsy in two young patients with lipoid nephrosis, was used as a control for immunofluorescence.

Antisera Used for Immunofluorescent Studies

The following antisera used for fluorescein labelling were kindly supplied by other investigators or purchased from commercial laboratories: antihuman IgG and antihuman C'lq (Dr. J. Morse and Dr. C. L. Christian [12]); antihuman IgA (Dr. R. D. Rossen [13]); antihuman β1C/β1A globulin (Hoechst Pharmaceuticals); anti-λ and anti-κ human light chains (Dr. E. R. Osserman [14]); antihuman fibrinogen (Dr. F. Gorstein); anti-horse globulin (Hyland Division of Travenol Laboratories, Inc.). The globulin fractions were separated from these antisera and from normal rabbit, goat, and horse sera and were conjugated with fluorescein by methods previously described.[15]

Tissue Processing for Light and Electron Microscopy

Each specimen was divided into three parts. The first portion was fixed in 10% neutral formalin, embedded in paraffin wax, sectioned, and stained with hæmatoxylin and eosin, periodic-acid Schiff, Weigert's for elastic counter-stained with hæmatoxylin and van Gieson, methyl-green pyronin, Gordon and Sweet's silver-impregnation method for reticulin fibres, and Perls' prussian-blue method for iron. The second part was fixed in buffered osmium tetroxide, embedded in 'Epon 812', sectioned, stained with lead citrate, and examined in a 'Philips EM 300' electron microscope. The third part was quickly frozen in a mixture of alcohol and dry ice or in isopentane slush in liquid nitrogen. Frozen sections 4μ thick were cut in a cryostat and treated with fluorescein-conjugated antibodies by techniques already described.[15]

Results

Morphological Changes

The histology of the lesions observed in most of these human liver transplants has been described elsewhere.[16, 17] The more striking morphological abnormalities are summarised in table ıı. A number of identifiable factors affected the structural findings; these included rejection, viral hepatitis, proved extra-hepatic biliary-duct obstruction, and recurrent carcinoma in the allograft.

The differentiation of rejection from other causes of abnormalities was difficult. On the basis of findings in untreated and treated canine liver allografts,[18-20] we assumed that dense infiltration of the portal tracts with large pyroninophilic lymphoid cells (fig. 1) indicated an active rejection process. This change was present in only two of the grafts, the 878-day specimen from OT 13 and the 68-day specimen from OT 16. Both livers also showed

obliterative arterial lesions. Cellular infiltration of moderate severity was the major abnormality in a third liver (OT 27). Arterial intimal thickening was present in a biopsy taken 304 days later from this same graft.

It was also assumed, on the basis of the same canine studies,[18-20] that a milder infiltration of the portal tracts with mostly non-pyroninophilic mononuclear cells, together with prominent centrilobular bile stasis, atrophy of the centrilobular hepatocytes, collapse and condensation of the central reticulin, and accumulation of fibrin and/or collagen fibrils in the spaces of Disse (fig. 2) probably indicated residual damage following subsidence of a rejection episode, either spontaneously or as the result of treatment. These changes were present in ten of the specimens (OT 8 biopsy, OT 22, OT 23, OT 25, OL 8 both biopsies, OL 18 second biopsy, OL 17, OL 19 both biopsies) (tables I and II).

In human and canine renal allografts recurrent clinical episodes of acute rejection or persistent chronic rejection are often associated with the development of intimal thickening of the arteries of the graft.[11, 22] Similar changes were present in the small branches of the hepatic artery in six of the liver allografts. In most of the specimens the arterial narrowing was accompanied by fibrosis, cellular infiltration, and cholestasis; in two livers there was also a portal type of micronodular cirrhosis (OT 9, OT 14).

Extrahepatic bileduct obstruction, with or without cholangitis, affected five of the liver specimens. The second graft in patient OT 13 was only given an arterial blood-supply, and at necropsy 19 days later contained many large infarcts. One graft (OT 29) showed evidence of viral hepatitis. This diagnosis was supported by positive serological tests for Australia antigen.

Two of the biopsy specimens examined were normal and showed no evidence of either active or past rejection. In one of these grafts (OL 10) cellular infiltration and centrilobular cholestasis, liver-cell atrophy, and reticulin collapse subsequently developed. The other patient (OL 9) remains well with normal liver function 2 years 8 months after transplantation.

Metastases from the primary liver tumour were present in the specimens from four of the grafts (OT 8, OT 14, OT 15, and OT 23).

There was no correlation between the severity of rejection and the degree of mismatching observed with tissue typing.

Immunofluorescent Findings

The controls were consistently negative. The findings in the twenty-six liver specimens are summarised in table III. Eleven of the samples gave negative results with all the fluorescent antibodies and fluorescent horse globulins. However, in two of these grafts (OT 9 and OT 16) there was arterial narrowing and a further three (OT 23, OL 8 first biopsy, OL 10 second biopsy) showed morphological evidence suggestive of residual damage caused by rejection.

The remaining fifteen specimens showed no significant binding of fluorescent antibody to x and λ light chains and equine globulins or to fluorescein-labelled horse globulins.

Nine (OT 25, OT 14, OT 8, OT 22, OT 27, OT 15, OT 8, OT 17, and OT 13) of the 15 specimens showed complement and IgG and/or IgM in the walls of blood-vessels. The sinusoids, small portal veins, and central veins (fig. 3) were most constantly affected, but in two grafts small hepatic artery branches (fig. 4) were also involved. The fluorescent pattern was interrupted linear, finely granular, or a combination of the two. Immunoglobulins were also present in the cytoplasm of infiltrating mononuclear cells (fig. 5) in eight of the nine liver samples. In eight of the specimens that contained immunoglobulins and complement there was morphological

evidence of active or resolving rejection. Extrahepatic bileduct obstruction and metastases were the main findings in the ninth specimen. The deposits of immunoglobulins and complement present in the biopsy of one liver allograft (OT 8) 100 days after transplantation were no longer detectable in the necropsy specimen 300 days later.

A further three liver samples showed IgG in the walls of their veins and sinusoids but lacked complement (OT 15, OL 17, and OL 19). Morphological evidence of rejection was present in two of the specimens; the third was dominated by bileduct obstruction and cholangitis.

IgA was demonstrated in only one of the specimens (OT 27); small deposits were present in the walls of the small arteries and sinusoids. The morphological evidence of rejection in this graft included obliterative arterial lesions.

Eight of the fifteen specimens with positive findings by immunofluorescence showed fibrinogen in the walls of the sinusoids (fig. 6) and veins. The deposits were associated with immunoglobulins in six specimens and with morphological evidence of rejection in seven.

Discussion

The purpose of this study was to establish whether immunoglobulins, complement, and fibrinogen were deposited in orthotopic liver allografts of patients treated with immunosuppressive agents, and to correlate the sites of localisation with the histological changes observed by light and electron microscopy.

Similar studies of kidney and heart allografts have already provided information which is useful in assessing possible pathogenetic mechanisms responsible for damaging the grafts. In kidney transplants appreciable deposits of immunoglobulins and complement in glomeruli and vessels were found in over half of long-surviving grafts.[5] In nearly all cardiac transplants studied, extensive deposits of immunoglobulins and complement were seen in the sarcolemma, muscle fibres, and coronary arteries.[10, 11] These observations suggested that kidney and heart allografts provoked strong humoral antibody responses in addition to delayed hypersensitivity, and provided evidence which linked deposition of immunoglobulins and complement with histological lesions and deterioration of graft function.

In contrast, our study of twenty-six specimens obtained at various times from nineteen human liver allograft recipients showed that binding of immunoglobulins and complement was less frequent and less intense than in kidney or hearts transplanted to patients who were treated with comparable immunosuppressive regimens. In fact, moderate amounts of immunoglobulins and complement were localised in the sinusoids and vessel walls in only four specimens. In five additional specimens only slight and focal binding was seen. The localisation of fluorescent antibodies to fibrinogen in the walls of sinusoids and vessels, observed in eight instances, probably corresponded to the fibrin deposits seen by electron microscope in Disse's space and in vessel walls.

These findings are in keeping with our previous studies with orthotopic liver transplantation in the dog.[20] In five animals, which received no immunosuppressive therapy, there were no deposits of IgG or complement in vessel walls at 4 days post-transplantation, and at 8 days only one allograft showed such deposits, whereas all the specimens had advanced morphological evidence of rejection. Paronetto et al.,[22] in their studies on untreated canine recipients of auxiliary liver allografts, also found that gammaglobulin did not appear in the walls of hepatic arteries until the second week after transplantation, but their conclusion was that humoral antibodies played a significant role in the destruction of allogeneic liver grafts. In rat auxiliary liver allografts, Lee and Edgington have showed somewhat earlier and more prominent immunoglobulin deposits.[24]

There are several possible explanations for the lack of correlation between the immunofluorescent findings and the morphological appearance of the allografts in some of the cases in this study. First, we cannot exclude the possibility that humoral factors may have participated in the production of tissue injury, because their presence in detectable amounts may be shortlived, as suggested by studies of sequential biopsies in human renal transplants [6] and also by observations on OT 8. In addition, the lesions in liver grafts do not progress uniformly, and sampling discrepancies may be frequent. However, although these two possibilities may deserve some consideration in explaining the absence of immunoglobulins and complement in rejecting liver allografts, other mechanisms are probably of greater importance. The findings suggest that the major injury is produced by cell-mediated immunity, since infiltrating mononuclear cells are frequently seen around the portal tracts and in Disse's space. Non-immunological factors such as sepsis, the use of hepatotoxic drugs, and viral hepatitis may also contribute to the liver damage.

Acknowledgments

Addendum

We have lately examined five additional orthotopic human liver allografts, 9 days, 1 month, 2½ months (two cases), and 3 years 5 months after transplantation (T. E. S.). The results accord with the reported findings. Even the 3 years 5 months allograft only showed minimal amount of fibrinogen products in the wall of a few sinusoids. Likewise in the other allografts immunoglobulins, complement, and fibrinogen were either absent or present in minimal amount only.

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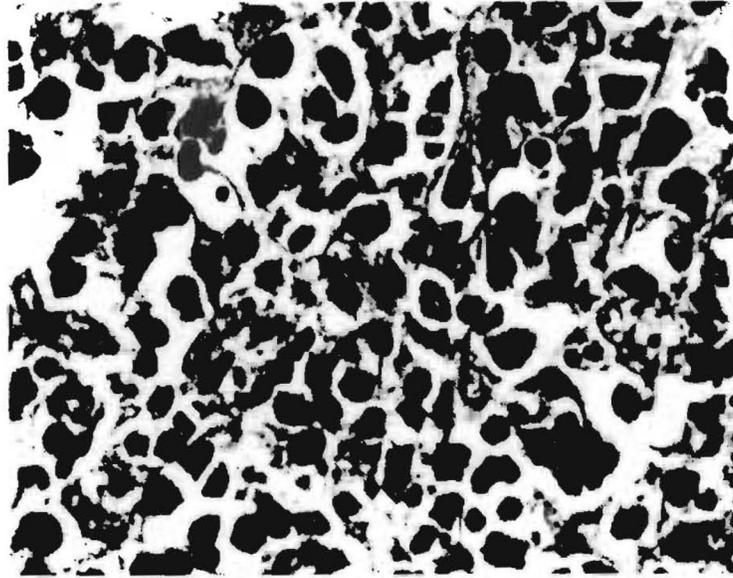
Fig. 1. Hepatic allograft (OT 16) removed 68 days after transplantation
Uncontrollable rejection followed early withdrawal of antilymphocyte globulin. Large number of infiltrating mononuclear cells with basophilic cytoplasm lies in a portal tract. (Hæmatoxylin and eosin; ×420.)
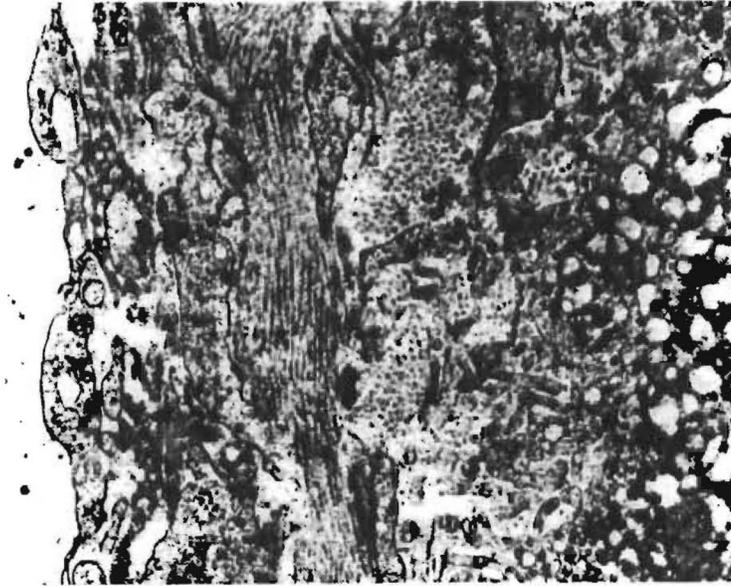
Fig. 2. Wall of sinusoid from hepatic allograft (OT 27) 514 days after transplantation
Fibrin and collagen occupy the space of Disse. The black granules are collections of glycogen in a hepatocyte. (Electron micrograph; ×12,000.)
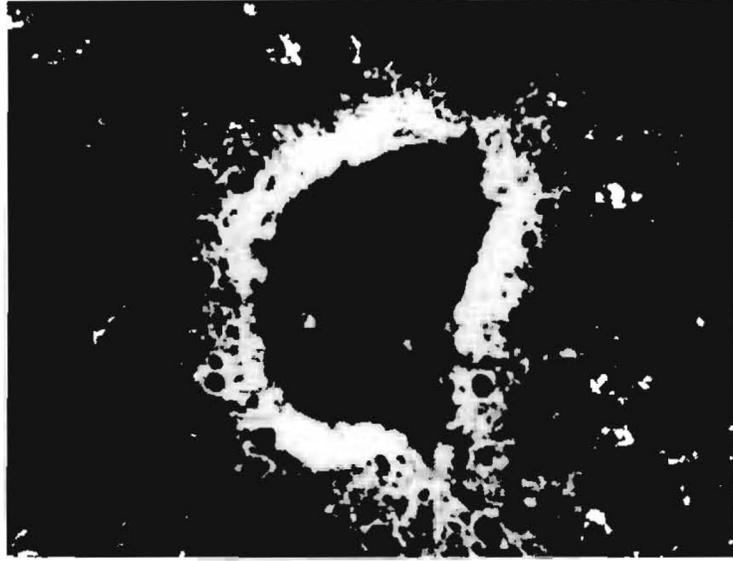
Fig. 3. Hepatic allograft (OT 14) 380 days after transplantation.
Localisation of fluorescent antibody to human IgG in the wall of a central vein. (×200.)
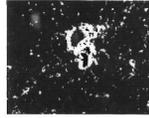
Fig. 4. Hepatic allograft (OT 25) 39 days after transplantation
Localisation of fluorescent antibody to human IgG in the walls of a vein and of a small artery.
(×250.)
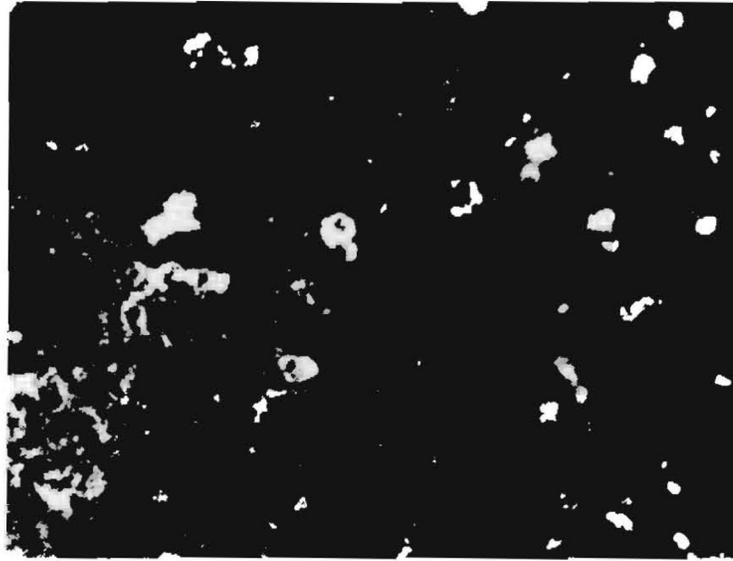
Fig. 5. Hepatic allograft (OT 25) 39 days after transplantation
The cytoplasm of infiltrating mononuclear cells is stained by fluorescent antibody to human IgG. (×300.)

Fig. 6. Hepatic allograft (OT 19) 968 days after transplantation
Localisation of fluorescent antibody to human fibrinogen in the walls of sinusoids. (×550.)

TABLE I

CLINICAL DATA ON 19 PATIENTS WITH ORTHOTOPIC HEPATIC ALLOGRAFTS

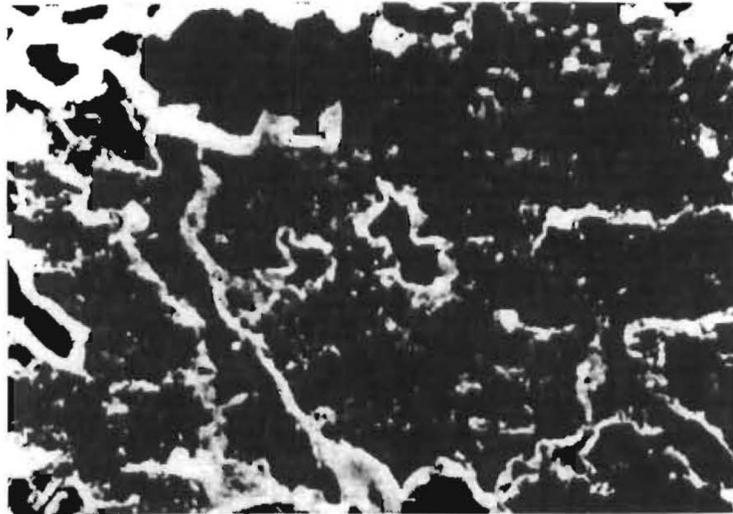| No.	Sex	Age	Disease	Sex	Age	HL-A group mismatches between donor and recipient	Specimen	Time specimen taken after transplant (days)	Clinical condition at time specimen collected
	Patient			Donor					
OT 8	F	1 yr. 7 mo.	Hepatoma	M	1 yr. 6 mo.	None	Biopsy	100	Plasma-bilirubin 3 mg./100 ml. Biopsy taken during operation for resection of extrahepatic metastasis. Rejection episode at 21 days had been reversed.
							Necropsy	400	Dead from carcinomatosis.
OT 9	F	1 yr. 9 mo.	Extrahepatic biliary atresia	F	4 yr.	HL-A2	Necropsy	133	Dead from liver failure caused by chronic rejection and septic infarct.
OT 12	F	1 yr. 4 mo.	Extrahepatic biliary atresia	M	1 yr. 2 mo.	HL-A6, HL-A7, Te 11	Necropsy	105	Dead from septic hepatic infarction. Rejection episode at 4 days had taken 70 days to reverse.
OT 13	M	2yr.	Extrahepatic biliaryatresia	M	3yr.	HL-A8, Te 11	Resected graft 1	878	Liver failure caused by chronic rejection.
				M	10 yr.	HL-A9, HL-A13 ± Te 6, Te 59	Graft 2 at necropsy	19	Dead from bacterial peritonitis. Graft only given arterial blood supply.
OT 14	F	16 yr.	Hepatoma	M	27 yr.	HL-A2	Resected graft 1	380	Liver failure caused by chronic rejection.
OT 15	M	44 yr.	Hepatoma, cirrhosis	F	20 yr.	Te 9	Necropsy	339	Dead from carcinomatosis. Rejection episode at 6 days had been reversed.
OT 16	M	1 yr. 11 mo.	Extrahepatic biliaryatresia	M	3yr.	None	Resected graft 1	68	Liver failure caused by chronic rejection.
OT 19	M	4 yr.	Intrahepatic biliaryatresia	M	10 yr.	HL-A2, HL-A3, HL-A7	Biopsy	968	Normal liver function. Rejection episodes at 29 and 72 days had been reversed.
OT 22	M	33 yr.	Cirrhosis	M	25 yr.	Te 12	Resected graft	10	Extrahepatic biliary duct obstruction.
OT 23	M	15 yr.	Hepatoma	M	6 yr.	HL-A6	Necropsy	143	Dead from carcinomatosis. Rejection episode beginning at 7 days had been reversed.
OT 25	M	45 yr.	Hepatoma	M	20 yr.	HL-A7	Necropsy	39	Bile peritonitis caused by bile fistula.
OT27	M	11 yr.	Hepatolenticular degeneration	M	11 yr.	Te 3 ±, Te9, Te 10, Te 12	Biopsy	210	Normal liver function.
							Biopsy	514	Normal liver function. Rejection episodes at 4 and 21 days had been reversed.
OT 29	M	6yr.	Intrahepatic biliary atresia	M	10 yr.	HL-A5, Tel 3	Biopsy	270	Viral hepatitis. Plasma bilirubin 2-3 mg./100 ml.
OL 8	M	57 yr.	Cholangio-carcinoma	M	52 yr.	4b	Biopsy	11	Acute rejection episode. Plasma bilirubin 10mg./100 ml.

| No. | Patient | | | Donor | | HL-A group mismatches between donor and recipient | Specimen | Time specimen taken after transplant (days) | Clinical condition at time specimen collected |
	Sex	Age	Disease	Sex	Age				
							Biopsy	46	Plasma-bilirubin 2·6 mg./100 ml. Rejection episode at 7 days had been reversed.
OL 9	F	44 yr.	Hepatoma	M	4 yr.	HL–A5, 4a	Biopsy	240	Good liver function. Rejection episode at 10 days had been reversed.
OL 10	M	56 yr.	Hepatoma, cirrhosis	M	37 yr.	HL–A7	Biopsy	38	Acute rejection episode. Plasma bilirubin 4 mg./100 ml.
							Biopsy	180	? rejection. ? cholangitis. Plasma bilirubin 8·7 mg./100 ml. Rejection episode at 38 days had been reversed.
OL 16	M	51 yr.	Cholangio-carcinoma	M	12yr.	HL–A10, HL–A12	Biopsy	17	Biliary peritonitis caused by bile fistula
							Biopsy	90	Liver function good. Biopsy taken during operation for relief of duodenal obstruction.
OL 17	M	47 yr.	Cholangio-carcinoma	M	23 yr.	HL–A2, 4b, LND	Biopsy	45	Liver function good. Biopsy taken during operation to deal with biliary leak. Possible rejection episode at 4 days had been reversed.
OL 19	M	28 yr.	Hepatoma	F	20 yr.	HL–A5, 4a	Biopsy	12	Acute rejection episode. Plasma-bilirubin 20 mg./100 ml.
							Biopsy	21	Rejection responding to treatment. Plasma bilirubin 15 mg./100 ml.

TABLE II

HISTOPATHOLOGICAL LESIONS IN 26 LIVER ALLOGRAFT SPECIMENS

Lesion	Patient and time (in days) when specimen was obtained																									
	OT 8	OT 8	OT 9	OT 12	OT 13	OT 13	OT 14	OT 15	OT 16	OT 19	OT 22	OT 23	OT 25	OT 27	OT 27	OT 29	OL 8	OL 8	OL 9	OL 10	OL 10	OL 16	OL 16	OL 17	OL 19	OL 19
	100	400	133	105	878	19	380	339	68	968	10	143	39	210	514	270	11	46	240	180	38	17	90	45	12	21
Mononuclear cell infiltration in portal tracts	+	+	0	±	++	0	±	+	++	±	+	±	±	+	±	++	±	0	±	+	0	+	±	±	±	±
Percentage of mononuclear cells with pyroninophilic cytoplasm	5	<5	0	0	40	0	0	<5	40	0	20	0	0	30	<5	5	10	0	0	10	0	10	5	0	0	0
Hepatic arteries — intimal thickening	0	0	++	0	+	0	+	0	+++	++	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0
Hepatic arteries—rupture of internal elastic lamina ..	0	0	0	0	+	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bileduct hyperplasia	+	+	0	++	0	0	±	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bileduct obstruction	0	++	0	++	0	0	0	++	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cholangitis	+	++	0	0	0	0	+	++	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0
Centrilobular cholestasis ..	+	++	++	+	+++	+	+++	±	+	0	+	+++	±	0	±	±	+	++	0	±	0	±	0	+	+	++
Focal areas of liver-cell necrosis	0	0	0	+	0	++	+	0	+	0	0	±	0	0	0	0	0	0	0	0	0	+	0	0	0	0
Infarcts	0	0	0	++	0	++	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centrilobular liver-cell atrophy and reticulin collapse..	0	+	+	+	0	0	++	++	±	0	0	+	++	0	0	0	+	+	0	±	0	0	0	+	0	0
Fibrosis	+	++	+	0	0	0	++	0	+	±	0	0	0	0	+	0	0	0	0	0	0	0	+	0	0	0
Cirrhosis	0	0	+	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fat in centrilobular hepatocytes	0	0	0	0	0	++	±	+	0	0	++	0	+	0	+	0	0	0	0	0	0	0	0	0	0	0
Metastases	+	++	0	0	0	0	+	++	0	0	0	+++	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE III

LOCALISATION OF FLUORESCEIN-LABELLED ANTIBODIES IN 26 LIVER ALLOGRAFT SPECIMENS

| Patient | Time specimen taken after transplant (days) | Deposits in liver specimens[*] | | | | | | Localisation of deposits within liver | | | |
| | | IgG | IgM | IgA | C' | | Fibrinogen | Walls of | | | Cytoplasm of mononuclear cells |
					β1C/β1A	C'1q		Arteries	Portal and central veins	Sinusoids	
OT 25	39	++	++	0	++	++	+	+	+	+	+
OT 14	380	++	+	0	0	+	++	+	+	+	+
OT 8	100	+	+	0	0	++	0	0	+	+	+
OT 22	10	+	+	0	+	+	0	0	0	+	+
OT 27	210	+	±	0	0	+	±	0	+	+	+
OT 15	339	+	0	0	0	0	0	0	+	0	0
OL 8	46	+	0	0	+	0	0	0	0	+	+
OL 17	45	+	0	0	0	0	0	0	0	+	0
OT 13	878	±	++	0	0	++	0	0	+	0	0
OL 19	12	±	0	0	±	0	++	0	0	+	+
OL 19	21	±	±	0	0	0	+	0	0	+	+
OT 27	514	0	0	±	0	0	+	+	0	+	0
OT 12	105	0	+	0	0	+	0	0	+	0	+
OT 19	968	0	0	0	0	0	++	0	0	+	0
OL 9	240	0	0	0	0	0	±	0	+	+	0
OL 8	11	0	0	0	0	0	0	0	0	0	0
OL 10	38	0	0	0	0	0	0	0	0	0	0
OL 10	180	0	0	0	0	0	0	0	0	0	0
OL 16	17	0	0	0	0	0	0	0	0	0	0
OL 16	90	0	0	0	0	0	0	0	0	0	0
OT 8	400	0	0	0	0	0	0	0	0	0	0
OT 9	133	0	0	0	0	0	0	0	0	0	0
OT 13	19	0	0	0	0	0	0	0	0	0	0
OT 16	68	0	0	0	0	0	0	0	0	0	0
OT 23	143	0	0	0	0	0	0	0	0	0	0

Patient	Time specimen taken after transplant (days)	Deposits in liver specimens [*]					Localisation of deposits within liver			
		IgG	IgM	IgA	C'			Walls of		Sinusoids	Cytoplasm of mononuclear cells
					β1C/β1A	C'1q	Fibrinogen	Arteries	Portal and central veins		
OT 29	270	0	0	0	0	0	0	0	0	0	0

[*] Intensity of immunofluorescence graded from 0 to 1 + i.