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POINT OF VIEW

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Chronic rejection of liver transplants revisited

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Abstract We examined 27 hepatectomy specimens to assess the frequency of foam cell endovasculitis and bile duct loss in chronic rejection. Arterial lesions, defined as total occlusion by subintimal foam cells and/or fibromuscular proliferation, were found mainly in hilar and septal arteries, whereas bile duct loss, defined as the absence of bile ducts in more than 50 % of portal tracts, affected mainly small tracts. Both were found in 20 livers (74 %). In two livers (7 %) there was significant bile duct loss but no arterial lesions, whilst in five cases (19 %) there were occlusive arterial lesions but no bile duct loss. Small arteries were involved in only 10 % of the cases. These results indicate that in one-third of the cases arterial and bile duct lesions develop independently of each other, suggesting different pathogenetic pathways. In addition, liver biopsy may not be pathognomonic since small arteries are involved in only 10 % of cases and bile duct loss may not be extensive. In such cases the diagnosis of chronic rejection should only be made in the presence of progressive clinical deterioration.

Key words Liver transplantation, chronic rejection · Chronic rejection, liver transplantation

Introduction

Orthotopic liver transplantation is a well established method of treatment for end-stage liver disease [19]. As worldwide experience has increased, technical problems responsible for early graft failure have been solved [4, 5]. In addition, advances in immunsuppressive therapy and in the treatment of postoperative complications have increased 1-year survival up to 90 % [4, 16, 17]. Nevertheless, rejection and infection remain the commonest causes of morbidity and graft loss in the 1st year after transplantation [10, 34]. Chronic rejection is responsible for at least 20 % of all graft failures in most medical centers [12, 14].

The biopsy interpretation of the morphological changes that take place in chronic rejection has assumed great importance in predicting irreversible graft damage [8, 26]. The two principal features of chronic rejection are occlusive arterial lesions and bile duct loss [7, 34]. Previous reports have indicated that these changes may occur together, or bile duct loss may occur alone, and hence some authors have used the term "vanishing bile duct" syndrome in lieu of chronic liver rejection [7, 9, 15, 18, 19, 20, 21, 31, 32, 34].

The aim of this study was to re-evaluate the accepted criteria for defining chronic rejection and to assess their significance in the diagnosis.

Materials and methods

Twenty-seven liver allografts that failed because of chronic rejection between 1980 and 1990 at the Cambridge/King's Hospital were reviewed. From each liver a total of eight blocks were examined. Samples (1.5 × 1.5 cm in size) were taken from the hilum, left, right, and median lobes (two samples from each). All sections were routinely stained with hematoxylin-eosin as well as reticulin, elastic van Gieson, periodic acid-Schiff (PAS) with or without diastase, orcein and Perls'.

Table 1 General data of transplanted patients with chronic liver rejection (*a1-ATD* a1-antitrypsin deficiency, *PBC* primary biliary cirrhosis, *EHBA* extrahepatic biliary atresia, *CAH* chronic active hepatitis, *HCC* hepatocellular carcinoma, *PSC* primary sclerosing cholangitis, *CrC* Cryptogenic cirrhosis, *HA* hepatitis A)

Case no.	OTL graft	Age (years)	Sex	Original disease	Graft survival (days)
1	533	10	M	a1-ATD	217
2	562	11	M	Wilson's	104
3	534	53	F	PBC	76
4	469	2	M	EHBA	133
5	440	30	F	PBC	160
6	366	3	M	a1-ATD	656
7	399	25	M	PBC	81
8	314	22	F	CAH	585
9	286	2	F	EHBA	218
10	259	10	F	a1-ATD	140
11	311	48	M	HCC	201
12	364	41	F	PSC	153
13	315	31	F	Wilson's	102
14	456	1	F	Tyrosinosis	136
15	454	55	F	PBC	110
16	257	1	F	EHBA	495
17	241	52	F	PBC	323
18	240	2	M	EHBA	195
19	235	42	M	CrC	117
20	234	26	F	PBC	26
21	606	16	F	CAH	167
22	195	37	F	PBC	87
23	175	18	M	Unknown	272
24	175	19	M	Unknown	636
25	168	22	F	HA	207
26	92	40	F	Budd Chiari	1284
27	148	11	M	NonA-NonB H	178

Arterial lesions were considered as diagnostic of chronic rejection in the presence of subintimal foam cell infiltrate and/or of fibromuscular proliferation. Bile duct loss was defined as the absence of bile ducts in more than 50 % of small portal tracts. Large arteries were considered those with a diameter of up to 0.3 cm, medium-sized arteries those less than 0.1 cm, and small ones those less than 0.01 cm

Results

Clinical findings

The general patient data are presented in Table 1. Of the 27 cases of chronic liver rejection, 11 were in males (one had two grafts) and 15 in females. The youngest patient was 13 months old and the oldest 55 years old. The mean age of the patients was 23 years and the median age 20 years.

Seven patients (one male and six female) had primary biliary cirrhosis (PBC), six (four male and two female) had metabolic diseases, four (two male and two female) had biliary atresia, five (two male and three female) had posthepatitic cirrhosis (including one case of cryptogenic cirrhosis), one patient (male) had hepatocellular carcinoma; one patient (female) had sclerosing cholangitis, one other male patient had Budd-Chiari syndrome, and yet another male patient had two liver transplants with unknown primary liver disease.

The mean graft survival was 261 days. The PBC patients had the shortest mean survival (123 days); mean survival without the PBC cases was 309 days. The longest graft survival was seen in the patient with Budd-Chiari syndrome (1284 days).

Pathological findings

The weight of the livers ranged from 900 to 2280 g (mean weight 1637.6 g). The external surface of the livers was smooth, with only two cases showing some nodularity. The cut surfaces were bile-stained with a greenish discoloration and occasionally with a nutmet appearance. The hilar vessels were prominent with thickened, yellowish walls.

Microscopically, in 25 of the 27 cases (93 %), the hilar and septal arteries showed variable degrees of narrowing or occlusion, due mostly to foam cell infiltration and sometimes to fibromuscular proliferation, with or without foam cell infiltration (Fig. 1). Two cases did not show any arterial changes. Hilar bile ducts were minimally affected. Only in one case was there complete replacement of the main bile duct and its branches by fibrous connective tissue. In six other cases there was moderate inflammatory infiltration.

In the small portal tracts, inflammation was absent or, at most, scanty. In 20 cases (74 %) bile ducts were absent

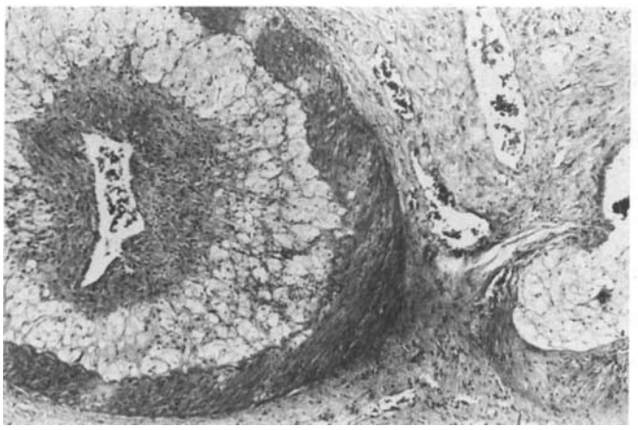

Fig. 1 Foam cell infiltration and minimal fibromuscular proliferation of a hilar artery

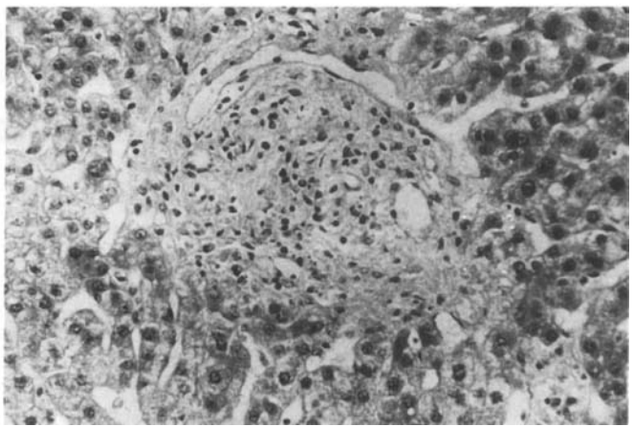

Fig. 2 Complete absence of bile ducts in a portal tract

from 50 %–100 % of the portal tracts counted (Fig. 2); in one case there was ductular proliferation. Another case showed ductular proliferation without bile duct loss, whilst six cases showed neither lesion.

Arterial obliteration with foam cell arteriopathy of small portal truct arterioles was seen in only three livers (10 %), and in two others there was significant arteriolar loss. These vessels were normal in 22 cases.

Eighteen livers (67 %) had both lesions (occlusive arterial lesions and bile duct loss), two livers (7 %) had bile duct loss but no arterial lesions, and in seven livers (26 %) there were occlusive arterial lesions but no bile duct loss (Table 2).

In analyzing the extent and severity of the arterial lesions (Table 2) it is apparent that the medium-sized hilar arteries were most commonly occluded ($n = 21$) or nar-

Table 2 Histological findings of bile ducts and arteries in hilar septa and small portal tracts [(*BDL* bile duct lesions, *Art L* Arterial lesions, *BDN* normal bile ducts, *BD Pr* bile duct proliferation, *Art Obl* Arterial obliteration (with foam cell arteriopathy)]

	Hilum and septa			Small portal tracts			
	BDL	Art L	BDN	BD Pr	BD Loss	Art Obl	Art Loss
1.	+	+	−	−	+	−	−
2.	−	+	−	−	+	−	+
3.	−	+	+	−	−	+	−
4.	−	+	−	+	−	+	−
5.	−	+	−	−	+	+	−
6.	+	+	−	−	+	−	−
7.	−	+	−	−	+	−	−
8.	−	+	−	−	+	−	−
9.	+	+	−	−	+	−	−
10.	−	+	−	−	+	−	−
11.	+	+	−	−	+	−	−
12.	−	+	−	−	+	−	−
13.	−	+	+	+	−	−	−
14.	−	−	−	−	+	−	−
15.	−	+	−	−	+	−	−
16.	+	−	−	+	+	−	−
17.	+	+	+	−	−	−	−
18.	−	+	−	−	+	−	−
19.	−	+	−	−	+	−	−
20.	+	+	−	−	+	−	−
21.	−	+	−	−	+	−	−
22.	−	+	+	−	−	−	−
23.	−	+	−	−	+	−	−
24.	−	+	+	−	−	−	−
25.	−	+	−	−	+	−	−
26.	−	+	+	−	−	−	−
27.	−	+	−	−	+	−	+
	7/27	25/27	6/27	2/27	20/27	3/27	2/27

Table 3 Correlation of graft survival days with arterial lesions (*OTL* orthotopic transplantation liver graft, *STEN* stenosed, *PTN* patent, *OCCL* occluded, *CAL* calcified)

No.	Case no.	OTL graft	Sex/age	Graft survival (days)	Size of arteries (hilum-septa)			Parenchymal arteries
					large	medium	small	
1	20	234	F 26	26	STEN	OCCL	PTN	PTN
2	3	534	F 53	76	STEN	OCCL	OCCL	OCCL
3	7	399	M 25	81	PTN	OCCL	PTN	PTN
4	22	195	F 37	87	OCCL	OCCL	OCCL	PTN
5	13	315	F 31	102	PTN	OCCL	OCCL	PTN
6	2	562	M 11	104	STEN	OCCL	OCCL	LOSS
7	15	454	F 55	110	PTN	OCCL	OCCL	PTN
8	19	235	M 42	117	STEN	OCCL	OCCL	PTN
9	4	469	M 2	133	STEN	OCCL	OCCL	OCCL
10	14	456	M 1	136	PTN	PTN	PTN	PTN
11	10	259	F 10	140	STEN	PTN	STEN	PTN
12	12	364	F 41	153	STEN	PTN	PTN	PTN
13	5	440	F 30	160	PTN	PTN	OCCL	OCCL
14	21	606	F 16	167	STEN	PTN	OCCL	PTN
15	27	148	M 11	178	NAR	PTN	OCCL	LOSS
16	18	240	M 1	195	STEN	PTN	OCCL	PTN
17	11	311	M 48	201	STEN	PTN	OCCL	PTN
18	25	168	F 22	207	STEN	PTN	OCCL	PTN
19	1	533	M 10	217	STEN	PTN	OCCL	PTN
20	9	286	F 2	218	OCCL	PTN	OCCL	PTN
21	23	175	M 18	272	OCCL	STEN	STEN	PTN
22	17	241	F 52	323	STEN	STEN	STEN	PTN
23	16	257	F 1	495	PTN	PTN	PTN	PTN
24	8	314	F 22	585	STEN	OCCL	PTN	PTN
25	24	175	M 19	636	STEN	OCCL	PTN	PTN
26	6	366	M 3	656	CAL	OCCL	OCCL	PTN
27	26	92	F 40	1284	CAL	OCCL	OCCL	PTN

rowed ($n = 3$) while only three cases were unaffected. In 20 cases the large arteries showed narrowing or complete occlusion; seven showed minimal or no changes. Small hilar arteries were completely occluded in 17 cases, narrowed in 3, and normal in 7. The small arteries or arterioles in the small portal tracts were mostly patent (22/27). The five cases that showed occlusion ($n = 3$) and loss ($n = 2$) were always accompanied by occlusion of the medium and small-sized hilar arteries.

In addition to the above microscopic findings, there were some nonspecific changes. In the hilum and septa there was chronic inflammation with fibrosis and scarring, as well as edema. In one case the inflammatory infiltrate consisted mostly of plasma cells and eosinophils. Occasionally, foreign body granulomas, due to suture material or to bile accumulation, were seen. A common finding was the presence of neural proliferation and, in two cases, neuritis. Hilar lymph nodes showed lymphocytic depletion in four cases, and in one case there was necrotizing lymphadenitis. In one case the main bile duct showed extensive squamous metaplasia with some epithelial atypia in the smaller branches.

The parenchyma showed bile stasis with feathery degeneration and cell loss around the central veins (Fig. 2). There was a variable degree of inflammation and occasional cell loss with centrolobular collapse. Two cases showed micronodular cirrhosis and one case nodular regeneration with hyperplasia.

Discussion

Analysis of our data reveals that in chronic rejection the arteries as well as the bile ducts are affected. However, arteries appear to be affected more commonly (93 %) than bile ducts (74 %). In addition, these data show that whilst chronic rejection is easy to recognize in the resected, failed liver graft, it is much harder to diagnose on biopsy [33]. This is due to the fact that the main lesion, foam cell arteriopathy, is most easily recognized in the large hilar arteries in the gross specimen, while the second diagnostic feature, bile-duct loss, is a microscopic feature located in trhe parenchyma. It might not be included in the liver biopsy, especially since by definition at least 50 % of the bile ducts should be absent for the diagnosis. The obliteration of the small arteries by foam cells in the small portal tracts is present in only 10 % of livers. In an additional 8 % of livers, complete arteriolar loss, although not generally considered as diagnostic, may indicate the presence of chronic rejection.

Our current data also show that in 33 % of livers arterial lesions and bile duct loss do not coexist, in contrast to previous reports suggesting the opposite [7, 34]. This finding may indicate that in one-third of the cases the two lesions may develop independently, suggesting the involvement of different pathogenetic processes.

It is likely that an immunologic mechanism is mostly responsible for the bile duct changes. As previous reports have indicated, the bile duct epithelium expresses a large number of adhesion molecules including class I and class II MHC, ICAM-1 (CD54) etc., thus becoming the target of T lymphocytes [1, 2, 25, 27, 28, 31, 32]. It should be noted that recent reports suggest that the so-called vanishing bile duct syndrome might be transient or reversible [15, 21].

On the contrary, the pathogenesis of the atherosclerosis-like lesions of chronic rejection remains as uncertain as in any other organ [24]. In the kidney, serial biopsies showed that the lesions followed repeated deposition of fibrin and platelet aggregates, which are then covered by endothelium [22, 23, 30]. Lipids liberated from platelets are then taken up by macrophages and smooth muscle cells [3, 11], both of which may acquire a foamy cytoplasm. Although the initial target is thought to be the endothelial cell, Billingham [3], in heart grafts, found little evidence of morphological injury to the endothelium.

Recently, immunologic mechanisms involving both humoral and cellular factors have been proposed. Deposits of antibodies and complement (IgM and Clq) have been detected in thickened blood vessels in liver allografts with chronic rejection [6]. In addition, a cell-mediated injury to smooth muscle cells, resulting in an inflammatory infiltrate to the outer media and aventitia, has been suggested as the main cause of accelerated atherosclerosis in heart grafts [13].

It is of interest that our data show that small parenchymal arteries or arterioles are less affected than large, medium, and small arteries in the hilum and septa. This may indicate a nonimmunologic process involving local hemodynamic factors affecting mostly the large arteries. In two cases with the longest graft survival (656 and 1284 days), the large arteries in the hilum showed severe arteriosclerotic changes with calcification of the arterial wall (Table 3). Only occasional propagation of foam cells to the small arteries or arterioles in the liver parenchyma was present.

In conclusion, our results indicate that arterial lesions are found alone in greater frequency than bile duct lesions, suggesting either different pathogenetic processes or divergent mechanisms of the same, probably immunologic, process. In addition, since small arteries are involved in only 10 % of the cases and bile duct loss may not be extensive, the diagnosis of chronic rejection should only be made in conjunction with clinical and biochemical data and in the presence of progressive clinical deterioration.

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