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Distribution of the MHC antigens after liver transplantation: relationship with biochemical and histological parameters

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SUMMARY

The aim of this study was to analyse the change in tissue distribution of HLA class I and class II molecules in patients during the course of liver transplantation. Sixty-one liver biopsies were analysed in 24 patients with or without rejection (acute or chronic) episode. In 93% of cases with chronic rejection and in 48% of cases with acute rejection episodes, HLA class I antigens are expressed in the membrane and/or the cytoplasm of hepatocytes. The expression of these molecules was significantly correlated with the ASP (aspartate amino transferase), AP (alkaline phosphatase), the bile duct damage and the centrolobular lesions. In contrast, the expression of HLA class II molecules is not correlated with either the type of rejection, the biological or histological findings. A strong expression of HLA class I antigen on hepatocytes after day 60 may be one of the best signs of chronic rejection.

Keywords liver transplantation MHC chronic rejection

INTRODUCTION

Generally, human liver transplantations generate rejection reactions at different levels. The clinical rejection is often controlled by the use of cyclosporine. Rejection of the transplanted liver seemed to involve mainly cell-mediated immunity (Starzl *et al.*, 1982). All variation in the HLA antigen expression could, therefore, be important because the efficient lysis of T lymphocytes requires the target cells to display these antigens on their membranes (Ascher *et al.*, 1983; Hall & Dorsch, 1984).

In normal liver, hepatocyte does not express HLA class I and class II molecules. On the contrary, the biliary cells are rich in HLA class I antigens; a very weak expression of HLA-DR antigens was demonstrated on cells of large ducts. In Kupffer cells, HLA-DR antigens are clearly expressed. The endothelial cells of blood vessels show HLA class I antigens, but HLA-DR are poorly expressed (Rouger *et al.*, 1986).

Nevertheless, these antigens may appear in some diseases (Barbatis *et al.*, 1981; Fukusato *et al.*, 1986). In the case of rejection, β-2 microglobulin and HLA- A, B and C molecules were shown to be strongly expressed on the hepatocyte membrane during the acute phase and tended to fall after the rejection episode (Nagafuchi *et al.*, 1985; Gugenhein *et al.*, 1987). HLA class II antigens have been also evidenced on biliary cells during rejection (Takacs *et al.*, 1983; Demetris *et al.*, 1985).

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The aim of this study was to analyse the changes in tissue distribution of HLA class I and class II molecules after liver transplantation; and to correlate this new distribution with the biological and histological findings.

MATERIALS AND METHODS

Patients

This study included 24 patients who were transplanted with an ABO-compatible liver. All patients were treated with cyclosporine; 61 biopsies were performed using a Menghini needle at the time of the transplantation and betwen day 5 and day 425 following liver transplantation.

Each specimen was divided, one part being fixed for routine light microscopic examination and the remainder embedded in tissue teck II mounting medium and snap-frozen in liquid nitrogen for immunofluorescence studies. In three cases where donors were DR7-positive and recipients DR7-negative, we tested the biopsies with a specific anti-DR7 in comparison with the non-polymorphic antibodies. These biopsies were performed at days 31, 56 and 88.

Definition of liver allograft rejection

Acute rejection was defined as requiring all three of the following criteria: biochemical abnormalities, histological abnormalities, and follow-up to exclude other causes of graft dysfunction. The clinical findings of weakness, fever, and

decreased bile output are non-pathognomonic. Biochemical abnormalities included increased (over baseline on two consecutive days) total serum bilirubin, alkaline phosphatase (AP), gammaglutamyltransferase (GGT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Histologic findings consistent with rejection included cellular portal infiltrate, bile duct damage, and endothelitis. Follow-up was continued for at least 8 weeks following the biopsy in order to observe evidence of rejection or response to anti-rejection therapy (three 1-g boluses of methylprednisolone on alternate days) and to exclude other causes of graft dysfunction.

Chronic rejection was defined histologically as the presence of obliterative endarteritis, cirrhosis, or marked bridging necrosis with paucity of bile ducts, and by the lack of response to therapy.

Immunoflourescence studies

Immunoflourescence procedure. The tissue samples taken from liquid nitrogen were immediately placed in a cryostat microtome at a temperature of −25°C. The cryostat-cut sections were of 4 to 6 μm. They were immediately transferred to glass slides and air-dried for 15 min at 15°C. The cryostat-cut sections were then kept at −80°C.

The sections were incubated for 30 min with monoclonal antibodies used with a predetermined dilution known to give an optimal performance. This dilution was determined by preliminary tests on tissue sections (kidney) and on cell suspensions (lymphocytes or erythrocytes). The sections were washed three times in a phosphate buffer solution, and then incubated with antiglobulin for 20 min at 20°C. The fluorescein-labelled antiglobulins used were anti-mouse antiglobulin (Meloy, diagnostic Pasteur). The sections were washed three times in a phosphate buffer solution. The slides were then mounted in 50% glycerol and examined with a Leitz Dialux fluorescent microscope. Several control reactions were used. The first consisted in incubation of the culture medium and the antiglobulin (reagent control). The second was the incubation of the tissue section with only the antiglobulin (antiglobulin control). The third was the examination of tissue sections used for autofluorescence phenomena (autocontrol).

In parallel to murine monoclonal antibodies, we used an immunofluorescence technique with biotinylated antiglobulins and avidin-coupled fluorescein (Vector Laboratories).

Antibodies. For each specificity studied several reagents were used. Tests for A, B, H, HLA-A HLA-B and HLA-DR antigens were performed with murine monoclonal antibodies. The production and characterization of monoclonal antibodies have previously been described (Mallissen *et al.*, 1982; Gane *et al.*, 1987; Mollicone *et al.*, 1987; Oriol *et al.*, 1987). The list of the monoclonal antibodies used is summarized in Table 1. A purified human polyclonal anti-DR7 (0920) (Institut Mérieux, France) was also used in three cases to distinguish between donor and recipient origins.

Statistical analysis

The association between increased hepatocyte or biliary cell fluorescence and each of the histological and biochemical factors considered was examined for statistical significance by χ^2 analysis with Yates's correction when necessary.

Table 1. List of monoclonal antibodies used (all are of murine origin)

	Code number	Remarks
ABH specificities		
Anti-A	21 W 1	Restricted spectrum
	31 W 1	Large spectrum
Anti-B	26 W 3	Restricted spectrum
Anti-AB	26 W 4	Reacts more strongly with A than with B antigens
Anti-H	MNS 210	Reacts specifically with H type 2
MHC specificities		
Anti-B_2 microglobulin	B.1.1.G6	
Anti-HLA-A, B, C	B.9.12	
Anti-HLA class I	B.1.23	Reacts with heavy chain
Anti-HLA-DR	B.8.11	

RESULTS

The patients were classified into three categories: patients without any rejection episode (five patients, 10 biopsies), patients with one or more rejection episodes (14 patients, 36 biopsies) and patients with chronic rejection (five patients, 15 biopsies).

A, B and H antigens

The histological distribution of A, B and H antigens was not modified by transplantation whatever the evolution was.

HLA class I antigens

The expression of these antigens on the 24 biopsies performed at the time of transplantation was not different from that described on normal specimens. HLA class I molecules were only evidenced on biliary cells, endothelial cells, Kupffer cells and dendritic cells.

Of the 61 biopsy specimens performed after transplantation, 32 (52%) saw a significant expression of HLA class I molecules on hepatocytes. These antigens were demonstrated in nine cases (15%) on the membrane only, in eight cases (13%) in the cytoplasm only, and in 15 cases (25%) on the membrane and in the cytoplasm of the hepatocytes; mainly in periportal but also in centroveinular areas. The distribution of the positive reactions was significantly different ($P < 0{\cdot}001$) in the three categories of patients previously described. Positive hepatocytes were observed in 14 of the 15 biopsies (93%) performed in patients with chronic rejection, in 10 of the 21 biopsies (48%) performed during acute rejection episodes and in eight of the 25 biopsies (32%) from non-rejected livers (Table 2). The expression of HLA class I antigens on the hepatocytes does not appear in early stages, of the nine sections from patients with acute rejection episode before day 10 (after transplantation), only one was positive. Furthermore, the strongest reactions were observed in biopsies performed in patients with chronic rejection (Table 3).

In contrast to the chronic rejection specimens, biopsies performed in patients with acute rejection are positive in seven out of 16 cases during biologically proven rejection reactions and tend to fall after rejection episodes.

Whatever the type of rejection, the expression of HLA class I molecules in the hepatocytes is significantly correlated with the

Table 2. Expression of HLA class I molecules in the hepatocytes according to the liver rejection

	Absence of any rejection (25 biopsies)	Acute rejection (21 biopsies)	Chronic rejection (15 biopsies)
Significant expression of:			
membranous HLA class I antigens	2 (0·08)	3 (0·14)	4 (0·27)
cytoplasmic HLA class I antigens	4 (0·16)	3 (0·14)	1 (0·07)
membranous and cytoplasmic HLA class I antigens	2 (0·08)	4 (0·19)	9 (0·59)
Absence of significant expression of HLA I molecules	17 (0·68)	11 (0·52)	1 (0·07)

Table 3. Biological, histopathological and immunofluorescence data of five patients with chronic rejection

												IF staining		
												HLA class I		HLA class II
	Time post	Histopathology				Laboratory values						HEP		
Patient no.	Tx (days)	PI	BL	VL	CLL	AST	ALT	AP	TB	DB	GPT	Mb.	Cyt.	BDE
38	7	2	1	1	0	1	4	2	14	44	NT	+	+	NT
	56	2	2	2	3	7	7	5	18	52	16	+	+	NT
	71	1	1	1	2	5	5	4	13	40	22	++	−	++
	76	2	2	2	3	4	4	4	13	40	17	++	−	++
	86	1	1	1	3	3	3	5	8	23	23	++	+	NT
	260	2	NT	1	1	2	1	4	1	4	NT	+	+	NT
	11	3	2	1	3	33	47	1	12	36	7	+	−	−
39	26	1	1	2	3	3	6	1	3	8	7	(+)	+	−
	51	1	0	0	1	8	12	1	6	18	10	(+)	(+)	−
	68	1	1	0	2	13	16	1	13	37	11	++	−	(+)
	200	NT	NT	NT	NT	14	10	2	6	18	18	++	−	−
	43	1	2	0	3	4	6	10	13	38	NT	++	+	+
49	58	1	1	3	2	1	1	1	1	2	5	++	+	++
	104	1	2	0	2	3	4	6	39	10	33	+	+	NT
33	112	2	2	1	1	15	22	4	2	8	28	++	++	−

The histologic findings were grading from 1 to 3 according to the severity of the lesions and the biological data were indicated as times of the normal value. The IF staining was graded from − to +++; −, negative; +, positive, very weak brightness; +, positive, very bright and always reproducible; ++, positive, strong brightness; +++, positive, very strong brightness. For biological parameters see Table 4.

BDE, bile duct epithelium; BL, bild duct lesions; CLL, controlobular lesions; Cyt, cytoplasm; HEP, hepatocytes; IF, immunofluorescence; Mb, membrane; NT, not tested; PI, portal infiltration; Tx, Transplantation; VL, vascular lesions.

AST and AP levels and with the bile duct damage and the centrolobular lesions (Tables 4 and 5).

HLA class II antigens

In contrast to normal specimens in which HLA class II antigens are only expressed on Kupffer cells, dendritic cells, and some endothelial cells, biliary cells of 25% of transplanted livers display HLA class II molecules. The expression of HLA class II molecules is not correlated with either the type of rejection, or the biological or histological findings.

In two of the three cases analysed with a specific anti-DR7, we have shown persistence of donor-specific class II molecules on endothelial cells at least 3 months after transplantation. The third sample was not interpretable.

DISCUSSION

The diagnosis of liver rejection is often very difficult to ascertain. Clinical, biological and histological data do not always provide unequivocal answers. So many authors try to define immunohis-

Table 4. Correlation between the expression of HLA class I antigens in the membrane of the hepatocytes and six biological parameters

Biological findings		Expression of HLA class I molecule in the membrane of the hepatocyte: Absence or very weak	Strong	χ^2
AST	<2	17	5	
(30 IU/l)	>2	20	19	$P<0.05$
ALT	<2	10	5	
(35 IU/l)	>2	27	19	NS
AP	<2	18	5	
(100 IU/l)	>2	19	19	$P<0.05$
TB	<2	16	7	
(20 Umol/l)	>2	21	17	NS
DB	<2	7	2	
(5 Umol)	>2	29	22	NS
GPT	<2	3	0	
(49 IU/l)	<2	24	20	

ALT, alanine aminotransferase; AST, aspartate aminotrnsferase; AP, alkaline phosphatase; DB, direct bilirubine; GPT, gammaglutaminyl transferase. <2, inferior to twice the normal level; >2, superior to twice normal level; NS, not significant; ( ), limitis of normal values.

Table 5. Correlation betwen the expression of HLA class I molecules in the membrane of the hepatocytes and the histologic findings

Histologic findings	Expression of HLA class I molecule in the membrane of the hepatocytes: Absence or very weak	Strong	χ^2 test
Cellular portal infiltrate			
Absence	6	1	
Presence	31	23	NS
Bile duct damage			
Absence	20	3	
Presence		19	$P<0.01$
Vascular lesions			
Absence	16	6	
Presence	21	17	NS
Centrolobular lesions			
Absence	22	8	
Presence	14	15	$P<0.05$

NS, not significant.

tological and prognostic criteria by studying the expression of MHC antigens in transplanted livers and other liver diseases. This study is in general agreement with earlier reports which have shown an enhanced expression of HLA class I antigens on biliary cells (Takacs *et al.*, 1983; Demetris *et al.*, 1985; Nagafuchi *et al.*, 1985).

However, our results show some discrepancies with those obtained by Nagafuchi *et al.* (1985), and Demetris *et al.* (1985). It is important to note that immunosuppressive therapy is able to influence the results. In the rat, it was shown that cyclosporine A completely suppressed the induction of HLA class II molecules, but not the induction of class I molecules. This observation, perhaps, explains why we have found a more significant number of specimens which express HLA class I molecules on the hepatocytes than those which express HLA class II molecules on biliary cells.

It is interesting to note that the expression of HLA class I antigens on the membrane of the hepatocytes (including specimens which were found positive on the membrane and in the cytoplasm) is correlated with some biological and histological data; in particular to the alkaline phosphatase enzyme level and to the bile duct damage. Several studies have shown that the dominant histopathological feature of hepatic allograft rejection is a progressive and destructive cholangitis (Starzl *et al.*, 1982; Takacs *et al.*, 1983). However, the significance of the expression of HLA class I molecules in the cytoplasm of the hepatocytes is not clear and the results were not correlated to any biological and histological data.

Detection of MHC markers is not very useful in confirming early acute rejection; a positive test generally signifies that the liver allograft could be chronically rejected. In our unit, we now consider that a strong expression of HLA class I antigen on hepatocytes after day 60 is one of the best signs of chronic rejection. However, expression of these determinants on the hepatocytes is not specific of allograft rejection and has also been observed in many other diseases involving bile duct obstruction (Fukusato *et al.*, 1986).

Our findings and this later observation together suggest that expression of HLA class I molecules on the hepatocytes is not involved in the 'first' rejection phenomena, but that it may be the reflection of secondary bile duct injury. Recently Innes *et al.* (1988) demonstrated in rats that cholestasis alone can increase the expression of class I MHC antigens on hepatocytes but has no effect on the expression of class II MHC antigens within the liver. Even if cholestasis is not able to initiate a liver rejection reaction, it is likely that intra-hepatic cholestasis, after liver transplantation, will contribute to any cytotoxic T cell activation.

Concerning the expression of DR antigens, our results seem to be in agreement with those of Rose *et al.* (1988) who studied 12 patients who were negative for DR7 but received heart from donors positive for DR7. They have shown persistence of donor-specific class II antigen on endothelial cells of the allograft human heart, 1 and 2 years after transplantation.

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