Published in final edited form as:

Transplantation. 1985 November ; 40(5): 504–509.

INDUCTION OF DR/IA ANTIGENS IN HUMAN LIVER

ALLOGRAFTS:

An Immunocytochemical and Clinicopathologic Analysis of Twenty Failed Grafts

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Abstract

Twenty failed human liver allograft specimens obtained at the time of retransplantation procedures were studied using a panel of monoclonal antibodies (T11, T4, T8, NK, B1, OKM1, OKM5, Ia, DR). A clinicopathologic analysis was used to distinguish between graft failures secondary to rejection (n=10) and those due, at least in part, to other causes (n=10). T lymphocytes constituted the major infiltrating cellular population in the liver in rejection cases, but significant numbers of B cells and monocytes/macrophages were present also. Following transplantation, but not before, the bile duct epithelium, as well as portal and central vein and hepatic artery endothelium expressed DR/Ia antigens. These structures are preferential targets of the rejection reaction. The selective destruction of bile ducts in livers undergoing rejection was manifested in these patients by striking elevations of serum gamma glutamyl transpeptidase (GGTP) activity, a marker of biliary epithelial damage. The induced expression of DR/Ia antigens on structures targeted for immune destruction may be an important event in the pathogenesis of liver allograft rejection.

The clinical diagnosis of rejection following liver transplantation is often one of exclusion. The pathologic interpretation of liver biopsy specimens obtained from graft recipients can be difficult (1). This difficulty in establishing a specific clinical diagnosis exists because the allograft is susceptible to a variety of insults. Presently, no definitive criteria for hepatic rejection are available other than various clinical parameters that can be combined with characteristic pathologic changes in biopsy specimens (1,2). Therefore, in an attempt to clarify at least some of the immunopathologic changes associated with liver rejection, we analyzed 20 failed allograft specimens utilizing a panel of monoclonal antibodies specific for surface antigens on inflammatory cells, and we combined this analysis with the patient's clinical and laboratory data. The histopathologic changes found in many of these posttransplant liver specimens have been reviewed in detail elsewhere (1).

MATERIALS AND METHODS

Case selection

Livers removed at transplantation were selected for this study because of the immediate availability of sufficient fresh tissue for analysis. Normal control liver tissue was obtained from two trauma cases and from three biopsy specimens performed for the detection of metastatic carcinoma in which no tumor was found.

Tissue preparation

Fresh liver tissue blocks were prepared within 1 hr of the resection or biopsy, frozen in OCT compound (VWR, Pittsburgh, PA) at -20°C in a cryostat, and stored at -60°C until sectioning.

Immunoperoxidase staining

Monoclonal antibodies were purchased from Becton-Dickinson, Inc., Sunnyvale, CA (Leu 7, HLA-DR); Ortho Pharmaceutical Corp., Raritan, NJ (OKM1, OKM5); and Coulter Electronics, Inc., Hialeah, FL (T11, T4, T8, B1, and I2). The chromogen, 3-amino-9-ethylcarbazole (AEC),⁵ and Mayer's hematoxylin were purchased from Sigma, St. Louis, MO. The reported specificities of the monoclonal antibodies used in this study are listed in Table 1. The blocks were equilibrated to -20°C over a 3-hr period, sectioned at $8\text{ }\mu\text{m}$ fixed in periodate-lysine-paraformaldehyde (PLP) (3) for 30 sec, washed in phosphate-buffered saline (PBS), and incubated in 0.1% hydrogen peroxide to block endogenous peroxidase activity. Appropriate dilutions of the monoclonal antibodies (determined on human tonsil tissue) were applied to the sections, incubated for 15 min, washed (in PBS), then incubated with peroxidase-conjugated rabbit anti-mouse immunoglobulins (1:20 dilution) (Accurate Chemicals) for 15 min. Following washing in PBS, the sections were incubated with AEC for 10 min, washed, counterstained with hematoxylin and mounted with Immunomount (Shandon, Sewickley, PA) for microscopy. In negative controls monoclonal antibodies were omitted and secondary antibody alone was applied, followed by the remainder of the procedure as outlined above.

Microscopic analysis

Serial histologic sections each stained with a different monoclonal antibody were reviewed without knowledge of the patients' clinical course. All cases were evaluated in sequence for each monoclonal antibody and subjectively scored (on a scale from 0 to 4). The stained inflammatory cells (T11, T4, T8, B1, OKM1, NK) present in each serial section were compared. A score of 0 indicated the number of inflammatory cells was indistinguishable from controls, and 4 the most intense infiltration by cells stained with a given monoclonal antibody when compared with all other cases. For example, the infiltrate illustrated in Figure 1f positive for OKM1 was scored as 4. Evaluation of the presence of DR/Ia (class II major histocompatibility complex [MHC]) antigens was also scored on a scale from 0 to 4 based on the relative number of bile duct epithelial, as well as hepatic artery and portal vein endothelial cells that stained on a review of the entire section. A score of 4 indicated all structures examined were stained. The patterns of the inflammatory cell infiltration and the localization of any particular mononuclear cell subset to the microscopic anatomy of the liver was noted.

⁵Abbreviations used: AEC, 3-amino-9-ethylcarbazole; GGTP, gamma glutamyl transpeptidase; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PLP, periodate-lysine-paraformaldehyde.

Clinical analysis

Information about the clinical course prior to retransplantation was obtained from a review of the charts with special attention focused on the etiology of graft dysfunction apart from rejection. The results of all investigative studies of biliary tract patency, blood and bile, bacterial, fungal and viral cultures, cyclosporine levels, and hepatitis serologies—as well as clinical impressions—were recorded. Maintenance immunosuppressive therapy in these patients consisted of cyclosporine and steroids. Response to bolstered immunosuppression of the events occurring immediately prior to resection were not taken into account, since the definitive therapy in these cases was allograft resection with retransplantation.

Clinicopathologic analysis

A final diagnosis was derived after a review of both the clinical course and pathologic specimen. Cases in which graft failure could be—at least Partially—attributable to causes other than rejection (group B) were separated from those in which rejection was the only apparent cause for the graft failure (group A). The patients in group A had no evidence of biliary tract obstruction, negative viral hepatitis B serologic tests, negative blood and bile cultures (when available), and a clinical diagnosis of rejection.

RESULTS

Tissue Distribution of Cellular Subsets

Normal control tissue—In normal liver tissue a small number of cells positive for T11, T4, T8, OKM1, and B1 antigens was found, mainly in the interstitium of the portal tracts and occasionally in the sinusoidal lumens. A similar distribution of inflammatory cells in normal liver tissue has been described previously (4).

DR/Ia staining was consistently seen in cells lining the sinusoids, and in the portal tracts in dendritic cells and in endothelial cells of small capillary-sized vascular channels. These dendritic cells and small capillaries were often in close association with bile ductules. Kupffer cells could not be reliably differentiated from endothelial cells within the sinusoids. Focal staining of central and portal vein endothelial cells was occasionally seen, but the majority of these cells did not stain. Biliary epithelial cells were negative for DR/Ia antigens.

The staining pattern with OKM5 was very similar to that seen for the DR/Ia antigens. OKM1 positivity was observed in these same locations with the exception that the staining was weaker in the dendritic and endothelial cells of small capillary-sized vessels found in the portal tracts.

Retransplanted specimens—The results of the clinicopathologic analysis are shown in Table 2, and the scoring of each cellular subset identified within the tissue specimens using the monoclonal antibody panel utilized is shown in Table 3. Group A (cases 1–10) consists of the cases that represent rejection, and group B (cases 11–20) had evidence of one or more causes other than rejection that could be, at least partially, responsible for the graft failure. It should be noted however, that coexistent rejection could not be ruled out in some of the cases in group B.

T cells (T11⁺) were the major population of infiltrative cells in the hepatic tissue of Group A (Table 3), and were most prominent in the portal tracts. They were also present in the centrilobular regions (cases 1–5), but they were fewer in number in this location. These cells were often located immediately beneath the portal and central vein endothelium, around and infiltrating the epithelium of small bile ductules (Fig. 1, a and b). Formation of tight cell clusters centered around bile ductules was noted in all cases in group A (Fig. 1c) and in some

cases (11,13,15, and 19) from group B. T cells were also prominent in group B in a distribution similar to that seen in group A. Infiltration and destruction of the biliary epithelium was more prevalent in group A specimens, making it difficult to identify small bile ductules—especially in cases 5, 7, 8, 9, and 10.

The ratio of T4/T8 cells was approximately equal to, or slightly greater than, one in all the cases—except case 10, in which the number of T8-positive cells was much greater than that of T4-positive cells. Both of these lymphocyte subpopulations and the biliary epithelium in the tissue specimens obtained from patients in group A.

B cells (B1⁺) were more conspicuous in group A specimens, forming small nodules (Fig. 1e) in cases 6, 7, 9, and 10 in the portal tracts. In Group B specimens no nodular aggregates of B1⁺ cells were found, but the cells were located in the portal tracts.

Monocytes/macrophages and polymorphonuclear leukocytes (oKM1⁺) were prominent in several cases from group B (Table 3), in which there was evidence for bacterial cholangitis and/or coexistent viral infection. In group A tissues, OKM1⁺ cells were evident immediately adjacent to bile ductules and in the vascular subendothelial space, but there were more T cells. However, they were conspicuous at the edge of the limiting plate, infiltrating the periphery of the lobule. NK cells formed a minor proportion of the cellular infiltrate in all the cases studied and had no apparent relationship to anatomic structures.

In contrast to the normal control specimens, biliary epithelium of both small interlobular and larger septal ducts, portal and central vein, and hepatic artery endothelium (200 μ m internal diameter) stained with anti-DR/Ia in allograft livers. The positivity was at times focal, in that it varied from portal tract to portal tract. In most instances, when the biliary epithelium was infiltrated by inflammatory cells, it was DR/Ia-positive. It should be noted however, that positive staining in the aforementioned structures was seen in both patient groups to varying degrees (Table 3). DR/Ia-positive infiltrating cells were not scored because of reactivity of anti-DR/Ia monoclonal reagents on several mononuclear cell subsets.

Correlation with liver injury tests—Bilirubin values varied widely, and no significant difference between the two groups was apparent. However, in group A patients the relationship of hepatocellular (SCOT/SGPT) to bile ductular (AP/GGTP) enzymes was indicative of a selective injury to the biliary epithelium (Table 2). Biliary tract patency was confirmed by cholangiography, or attested to by ultrasound or computerized axial tomography in all cases from group A. No significant differences in medication regimens was noted between the two groups. The hepatocellular enzymes were markedly elevated in the serum of the patients in group B in whom significant hepatocellular necrosis was apparent microscopically.

DISCUSSION

An attempt was made to segregate the cases in which the only apparent cause for graft dysfunction and eventual failure was allograft rejection (group A) from those in which other causes of graft failure were possible. It was recognized that when we compared the findings in group A with those in group B that the two groups differed with respect to time posttransplant and incidence of primary disease, that selection based on allograft failure introduced bias, and that some cases in group B may have had a component of rejection. However, the spectrum of primary disease in cases included in this study in general reflects that seen in this transplant population. Also, it is not uncommon for patients with sclerosing cholangitis and biliary sepsis pretransplant to develop septic cholangitis post-operatively (1).

We are not suggesting therefore, that all experiencing rejection will fit the profile of the patients we studied in group A.

Although T cells are the major subpopulation of inflammatory cells present in the rejecting liver, macrophages and B lymphocytes are a significant proportion of the infiltrate. Thus, it is likely that several immune mechanisms contribute to graft destruction, as suggested by Hayry (5). In this respect, the composition of the inflammatory infiltrate in group A specimens is not unique to rejection (4). However, the localization of the infiltrate beneath the endothelium of veins and the formation of tight clusters of T11⁺ (Fig. 1b) and OKM1⁺ cells centered around, and infiltrating, small bile ductules suggests that a selective immunologically mediated reaction may be directed at these structures. This phenomenon may be related to the differential expression of class I and II MHC antigens within the liver.

The portal tracts normally contain an abundance of DR/Ia-positive dendritic cells and small capillary-sized vascular endothelial cells, as shown in the control specimens used in this and in other studies (6). Expression of class I and II (MHC) within the liver is normally more prominent on endothelial, reticuloendothelial, and biliary epithelial cells (mostly class I), than it is on hepatocytes where it is either weak or absent (6-8). It appears that, following transplantation, DR/Ia antigens become expressed on venous endothelium, and focally on biliary epithelium and hepatic artery endothelium. This adds greatly to the immunogenic potential of these structures (5,9). Consistent with this concept is the finding that morphologically the structures expressing these DR/Ia antigens are preferentially involved in the rejection reaction (1,2,10). Porter was the first to document the swelling of the portal and central vein endothelial cells associated with subendothelial inflammatory cells in untreated animal allograft recipients (2). Following successful transplantation, Porter also documented the replacement of sinusoidal Kupffer cells in human allografts by host reticuloendothelial cells (2). The above observations indicate that the preferential, but not exclusive, localization of the inflammation associated with rejection to the portal tracts may be related to the presence and concentration of antigen in those areas.

Focal DR/Ia positivity in biliary epithelial cells has been reported recently in one orthotopic human transplanted liver (11), in graft-versus-host disease in experimental animals (12), and in primary biliary cirrhosis (13). Bile ducts are destroyed in each of these disorders (1,10,14-16). It is interesting to note that GGTP is located in the biliary epithelial cells and in animal studies has been shown to be preferentially elevated when there is selective injury to bile ducts (17). We propose that the selective immunologically mediated destruction of biliary epithelium in rejection is manifest in patients by striking elevations of serum GGTP (18) in comparison with serum hepatocellular enzymes. A similar hypothesis has been suggested in the pediatric liver allograft recipients (19).

It appears that the induced expression of DR/Ia antigens on biliary epithelium occurs to some degree in most, if not all, transplanted liver allografts because positive staining was seen in the tissue obtained from patients in both groups. Whether this expression is a result of ongoing immunologic reaction with local production of lymphokines, circulating mediators involved in the alloreaction, or simply cell damage and regeneration is uncertain. It has been emphasized, however, that the increased expression of DR/Ia antigens occurs in lesions involving activated lymphocytes (12). Although no clear-cut difference with respect to DR/Ia antigen expression exists between these two groups, comparison of rejection specimens with posttransplant biopsies in which there is no complicating pathological problem (unlike group B) may yield more informative data. Likewise, staining of liver specimens for the presence of DR/Ia antigens removed secondary to toxic injury alone without immune mediated destruction may help determine whether lymphokines are involved in inducing the expression of these class II antigens on biliary epithelial cells.

The significance of the inducible expression of DR/Ia antigens on structures targeted for immune destruction is open to speculation. It has been reported however, that class I and II histocompatibility antigens are capable of eliciting a cytolytic T lymphocyte response, and that antigen density on the target cell may be a factor in recognition (5,9). The allograft reaction with inflammatory cell infiltration may be triggered by structures in the portal tracts that normally express both class I and II MHC antigens. The initial events may then be followed by induced expression (possibly via lymphokines) of class II antigens on nearby structures, making them more susceptible to immune recognition and destruction. Hall et al. (20) reported induced expression of DR antigens in the kidney tubular epithelial cells during rejection of renal transplants. He suggested that expression of DR antigens may be important in enhancing the capacity of these cells to be recognized by a delayed-type hypersensitivity rejection reaction.

We cannot comment on the reversibility of DR/Ia expression with treatment of rejection because no samples from the same patient were examined sequentially. Nevertheless, interruption of the processes associated with DR/Ia antigen expression on bile ducts may enhance graft survival, since destruction of these cells appears to be a significant contributing factor in graft failure.

The limitations of an in-situ analysis of inflammatory infiltrates using monoclonal antibodies in renal transplantation biopsy specimens has been recently outlined by Hancock (21). They include the specificity of the antibody-antigen reaction, distribution and alteration of antigen expression in mononuclear cells depending on their maturity or activity, and the correlation of the phenotype with the functional properties of the cell. These particular limitations also apply to our study. Nevertheless, we think that the information gained from this in-situ analysis offers an insight into at least some of the immunogenetic mechanisms potentially associated with the initiation and/or maintenance of hepatic rejection.

Acknowledgments

The excellent technical assistance of Mrs. Arlene Ambro-Luther is acknowledged. We also recognize Drs. M. Nalesnik and D.G. Sheahan for helpful advice, and Mrs. Mary Kessler for preparation of the manuscript.

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Figure 1.

(a) T lymphocytes (T11⁺) in the portal tract from case 1. Note the cells beneath the portal vein (PV) endothelium (large arrowhead), surrounding bile ductules (arrow), and relative restriction of T11⁺ cells to the portal tracts (T11 IPEX, hematoxylin; $\times 125$). (b) Higher power (500 $\times$) of the above specimen showing T cells adjacent to and infiltrating a small bile ductule sectioned longitudinally (out-lined by arrows). (c) T cell lymphocytes (T11⁺) in liver tissue from case 4 showing tight clustering of inflammatory cells surrounding a small bile ductule (arrow, 250 $\times$). (d) Liver tissue from case 2 stained for Ia antigen, showing positivity in larger septal bile duct (BD), endothelium of hepatic artery (HA, arrowhead) and small bile ductules (arrows), which are surrounded by inflammatory cells (125 $\times$). (e) B lymphocyte (B1⁺) in a portal tract from case 7 showing a nodule of B cells. Bile ductules could not be seen in this portal tract (315 $\times$). (f) OKMI⁺ cells in tissue from case 13 showing numerous positive cells in the portal tracts. This case was complicated by sepsis and bacterial cholangitis (125 $\times$).

Table 1

Listing and specificity of monoclonal antibodies used

Monoclonal Ab	Antibody specificity
T11	Total T cells
T4	T helper/inducer subset ^a
T8	T suppressor/cytotoxic subset
OKM1	Monocytes, some endothelial cells, granulocytes
OKM5	Adherent monocytes, platelets, some endothelial cells
B1	B lymphocytes
12(Ia)/DR	Activated T cells, B cells, monocyte/macrophages, cells expressing class II MHC antigens
Leu 7(NK)	Natural killer cells, null cells

^aCrossreacted in some cases with sinusoidal cells in liver.

Clinicopathologic data from liver allograft patients

Table 2

Case	Age/Sex	Original disease	Time Post-TX. (days)	Clinicopathologic interpretation ^a	Laboratory values ^b				
					BIL (T/D; mg/dl)	SGOT (U/ml)	SGPT (U/ml)	AP (IU/L)	GGTP (IU/L)
1	25/F	CAH ^c	8	Rejection	9.8/6.6	130	112	44	66
2	42/F	PBC	18	Rejection	19.4/13.6	70	337	715	2360
3	51/F	PBC	30	Rejection	39.6/33	65	153	579	1134
4	23/F	CAH	41	Rejection	28.2/13.4	85	46	990	708
5	44/F	PBC	70	Rejection	19.8/12.7	563	1044	956	1641
6	44/F	PBC	93	Rejection	27.0/21.0	412	552	1008	>3000
7 ^d	44/F	PBC	150	Rejection	15.2/10.1	210	274	896	803
8	22/F	2°BC	240	Rejection	10.2/7.6	121	177	990	1455
9	19/F	CAH	2190	Rejection	20/16.8	307	241	1365	1045
10	32/M	CHF	>4380	Rejection	5.5/3.0	124	98	792	2660
11	25/M	Toxin ^e	8	Possible circulating toxin renal failure; hypotension/cholangitis	27.2/22	607	369	NA	221
12	39/M	SC	8	Poor graft preservation	18.6/15.2	70	346	80	67
13	32/M	SC	10	<i>Klebsiella</i> sepsis/bacterial cholangitis	3.7/1.6	1216	1530	118	32
14	29/M	SC	14	Biliary tract obstruction	31.8/24	4116	3969	122	82
15	32/M	SC	19	MI ×2/sepsis/graft ischemia	15.0/12.7	3240	4590	498	NA
16	21/F	CAH	19	Hepatic artery thrombosis/graft ischemia	8.8/5.3	135	379	136	163
17	39/F	PBC	22	Coagulopathy/renal failure/cyclosporine >2000 mg/ml	25.8/19.8	139	199	319	320
18	26/M	SC	40	Treated rejection and CMV	5.2/4.1	5479	1655	154	119
19	45/M	SC	41	Sepsis/rejection and CMV	22.4/18.4	81	27	86	156
20	32/M	SC	48	Treated rejection and CMV	5.1/4.1	972	665	81	60

^aPertinent negatives in cases 1–10: biliary tract and blood vessel patency, hepatitis serologies, blood and bile cultures (when available).

^bBilirubin (total/direct), serum glutamate oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (AP) gamma glutamyl transpeptidase (GGTP).

^cAbbreviations: CAH = chronic active hepatitis (cause uncertain); PBC = primary biliary cirrhosis; 2° BC = secondary biliary cirrhosis; CHF = congestive heart failure; CMV = cytomegalovirus hepatitis; MI = myocardial infarction; NA = not available.

^dCase 7 is the second failed allograft from the patient in case 5.

e Toxin exposure (2-nitropropane) (22).

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Table 3Analysis of inflammatory cell subsets^a in failed allograft specimens

Case no.	Portal infiltrate			DR/ta staining		
	T cells (T11)	B cells (B1)	Monocyte/macrophage polys (OKM1)	BDE ^b	HAE	PVE
1	3	1	2	4	3	3
2	3+	2	2	4	3+	3
3	2+	1	2	2+	2+	3
4	3+	1	1	3+	4	3+
5	2+	1	+	— ^d	3	4
6	3+	2	2	— ^d	2+	3
7	3	2	1	— ^d	3	2+
8	1+	+/-	+/-	1	2	2
9	3+	2	1	— ^d	+/-	+/-
10	3+	2	1+	— ^d	1	1
11	1+	+/-	2+	1	+/-	1
12	1	+/-	2+	+/-	+/-	1
13	3	1+	4	— ^d	3	3
14	3	1+	4	2	1+	3
15	1+	1	1+	2+	3	3
16	2+	+/-	1	3	2+	3
17	+/- ^c	0	0	0	0	+/-
18	2	+/-	2+	4	2+	3
19	3	1+	1+	+/-	1+	1+
20	3	2	2+	2+	2	3

^aSee the *Materials and Methods* section.^bAbbreviations: BDE = bile duct epithelium; HAE = hepatic artery endothelium; PVE = portal vein endothelium.^c+/- = Slightly greater than control tissue.^dThe bile ductules were decreased in number and/or obscured by inflammation, making scoring difficult.