Transplant Int (1992) 5 [Suppl 1]: S629–S630

TRANSPLANT
International

Non specific increased expression of class I major histocompatibility complex (MHC) antigens on rat liver grafts

J. Gugenheim[1], L. Amorosa[1], B. Fabiani[2], I. Astarcioglu[3], M. Gigou[3], F. Crafa[1], M. Reynes[2], H. Bismuth[3]

[1] Laboratoire de Recherches Chirurgicales et Service de Chirurgie Digestive, Hôpital Saint-Roch, B. P. 31906006 Nice Cedex
[2] Laboratoire d'anatomie Pathologique, [3] Unité de Chirurgie Hépatobiliaire, Hôpital Paul Brousse, 94800 Villejuif, France

Major histocompatibility complex (MHC) antigens play a major role in the rejection reaction and their increased expression may increase the host response to the foreign graft [1]. Several clinical [2–5] and experimental studies [6, 7] have demonstrated increased expression of MHC antigens on the different cell components of liver allografts during rejection. However modified expression of MHC antigens may also occur in certain liver diseases [8–10], after cholestasis [11] or on a regenerating liver [11]. In this experimental study in inbred rats, we compared the expression of MHC antigens on liver cells during rejection and non-immunological situations (cholestasis, cytolysis, regeneration).

Key words: MHC antigens – BN rats – Rejection

Animals and methods

Rats. Inbred rats of the following strains were purchased from CNSEAL (Orleans La Source, France): Brown Norway (BN) ($RT1^n$), DA ($RT1^a$).

Experimental protocol

There were seven groups of BN rats in this study. In group 1, liver from DA donors were grafted into BN recipient rats and biopsies were carried out on days 5, 8 and at time of death. In group 2, liver isografts were carried out in BN rats and biopsies were performed at days 5, 10 and 15. In group 3, cholestasis was induced by bile duct ligation and rats were sacrified 21 days later. In group 4, cytolysis was induced by the injection of galactosamine and rats were sacrificed 48 h later. In group 5, normothermic ischemia was induced by a 90 min occlusion of the portal pedicle and rats were sacrified 30 days later. Group 6 consisted of BN rats with a 70% hepatectomy, sacrificed 48 h later. Group 7 consisted of control BN rats.

Offprint requests to: J. Gugenheim, Laboratoire de Recherches Chirurgicales et Service de Chirurgie Digestive, Hôpital Saint-Roch, B. P. 31906006 Nice Cedex

Surgical procedures

Liver transplantation. Orthotopic liver transplantation was performed using cuff techniques for the portal vein, infrahepatic vena cava and biliary anastomoses, as described by Kamada [13]. Ischemia times were in the range of 20–30 min.

Induction of cholestasis. Biliary obstruction was induced by a double ligation of the common bile duct with a non-resorbable suture (7-0 silk). The common bile duct was then transected between the ligatures to prevent recanalization. After closure of the abdominal incision, rats were allowed to recover. Rats were killed 21 days post-ligation.

Ischemic-induced cytolysis. A temporary normothermic ischemia of the liver was induced as follows: the hepatic pedicle was occluded for 90 min with a microvessel clip. Rats were sacrificed 30 days after the end of the occlusion.

Galactosamine-induced cytolysis. Rats were given galactosamine 1.2 mg per kg body weight intraperitoneally. Rats were sacrificed 48 hours later.

Study of regenerating liver after partial hepatectomy. Fast growth of the liver was provoked by the removal of two-thirds of the total liver mass, according to the method described by Higgins and Anderson [14]. Animals were sacrificed 48 h later.

Histological and immunohistological studies

Hematoxylin-eosin stain and Masson trichrome were used for conventional histological examination. For immunohistological studies, a peroxydase antiperoxydase method using mouse monoclonal antibodies to rat MHC antigens (MRC OX27 for class I and MRC OX 17 for class II AG) was carried out as described by Fabiani et al. [7].

Results

Expression of class I MHC antigens (Table 1)

In control livers, there was no detectable class I antigen on the hepatocytes. Positive staining was seen on sinusoidal lining cells and was not modified in experimental groups.

Table 1. Expression of class I MHC antigens on rat hepatocytes

Groups	Grades of staining				
	n	0	+	+	+ + +
1. Allografts	5	0	0	3	2
2. Isografts	5	1	4	0	0
3. Bile duct ligation	5	0	0	3	2
4. Galactosamine cytolysis	5	0	0	1	4
5. Ischemic cytolysis	5	0	0	1	4
6. 70% hepatectomy	5	2	3	0	0
7. Control	5	5	0	0	0

In the isografts, weak (+) class I induction on hepatocytes and biliary cells was noted on days 5, 10 and 15. In DA to BN allografts, strong (+ +) induction of class I Ag was seen on hepatocytes on days 5, 8 and at time of death. A similar induction was seen in rats with cholestasis. A very strong (+ + +), induction of class I Ag was noted in rats with galactosamine and ischemic-induced cytolysis.

Expression of class II MHC antigens

No expression of class II antigens was seen on hepatocytes in any of the specimens studied. Induction of expression of class II antigens was seen only on biliary epithelium and on sinusoidal cells after liver allografting (group 1).

Discussion

In this study, we did not observe any expression of class I antigens on the hepatocytes of normal non-transplanted livers. This result is in line with several experimental [15] and clinical [16] studies, but the possibility that there is a low level of expression below the limit of sensitivity of the immunodetection method cannot be excluded.

This study demonstrated the induction of expression of MHC class I antigens on hepatocyte membranes during rejection of liver allografts. Isografts also became class I positive, though to a lesser extent than allografts. A massive induction of class I antigens was observed after cholestasis, galactosamine-induced or ischemic cytolysis. Alternatively, class II induction on biliary epithelium and sinusoidal cells appeared to be specific for allograft rejection. Induction of class I antigens on tissue that was previously class I negative may have some important consequences for T cell cytotoxicity. It is known that class I expression is necessary for cytotoxic T cells to recognize and lyse virally-infected cells [1] or tissues bearing alloantigens [17]. Increased hepatocyte MHC class I antigen expression may increase susceptibility of hepatocytes to lysis by cytotoxic T lymphocytes [1]. This may explain the significantly higher incidence of rejection observed after severe preservation injury [18]. In fact cholestasis, ischemic and toxic cell damage, and regeneration are frequently present after liver grafting. All these conditions may contribute to an increased sensitivity of liver allografts to rejection.

References

1. Zinkernagel RM, Doherty PC (1979) MHC cytotoxic T cells: studies on the biological role of polymorphic transplantation antigens determining T cell restriction-specificity, function and responsiveness. Adv Immunol 27:51
2. Demetris AJ, Lasky S, van Thiel DH, Starzl TE, Whiteside T (1985) Induction of DR/IA antigens in human liver allografts: an histocytochemical and clinopathologic analysis of twenty failed grafts. Transplantation 40:504–509
3. So SKS, Platt JL, Ascher NL, Snover DC (1987) Increased expression of class I major histocompatibility complex antigens on hepatocytes in rejecting human liver allografts. Transplantation 43:79–85
4. Gugenheim J, Rouger P, Gane P, Capron-Laudereau M, Reynes M, Bismuth H (1987) Expression of blood group antigens including HLA markers on human liver allografts. Transplant Proc 19:223–225
5. Rouger P, Gugenheim J, Gane P, et al. (1990) Distribution of the MHC antigens after liver transplantation: relationship with biochemical and histological parameters. Clin Exp Immunol 80:404
6. Settaf A, Milton AD, Spencer SC, Houssin D, Fabre JW (1988) Donor class I and class II major histocompatibility antigen expression following liver allografting in rejecting and non-rejecting rat strain combinations. Transplantation 46:32–40
7. Fabiani B, Astarcioglu I, Gugenheim J, Tricottet V, Bismuth H, Reynes M (1989) Expression of major histocompatibility complex antigens on vascular endothelium of spontaneously tolerated liver allografts. Transplant Proc 21:407–408
8. Fukusato T, Gerber MA, Thung SN, Ferrone S, Schaffner F (1986) Expression of HLA class I antigens on hepatocytes in liver diseases. AM J Pathol 123:264
9. Ballardini G, Bianchi FB, Doniach D, Mirakian R, Pisi E, Bottazzo GF (1984) Aberrant expression of HLA-DR antigens on bile duct epithelium in primary biliary cirrhosis: relevance to pathogenesis. Lancet I:1009
10. Barbatis C, Woods J, Morton JA, Fleming KA, Mc Michael A, Mc Gee JOD (1981) Immunohistochemical analysis of HLA (A, B, C) antigens in liver disease using a monoclonal antibody. Gut 22:985–991
11. Innes GK, Nagafuchi Y, Fuller BJ, Hobbs KEF (1988) Increased expression of major histocompatibility antigens in the liver as a result of cholestasis. Transplantation 45:749–752
12. Jonjic S, Radosevic Stasic B, Cuk M, Jonjic N, Rukavina D (1987) Class II antigen induction in the regenerating liver of rats after partial hepatectomy. Transplantation 44:165–168
13. Kamada N, Calne RY (1979) Orthotopic liver transplantation in the rat: technique using cuff for portal vein anastomosis and biliary drainage. Transplantation 28:47
14. Higgins GM, Anderson RM (1931) Experimental pathology of the liver. Arch Pathol 12:1986
15. Lautenschlager I, Hayry L (1981) Expression of the major histocompatibility complex antigens on different liver cellular components in rat and man. Scand J Immunol 14:421
16. Franco A, Barnaba V, Natalio, Balsano C, Musca A, Balsano F (1988) Expression of class I and class II major histocompatibility complex antigens on human hepatocytes. Hepatology 8:449–454
17. Mc Michael AJ, Ting A, Zweevink HF, Askonas BA (1977) HLA restriction of cell mediated lysis of influenza virus infected human cells. Nature 270:524
18. Howard TK, Klintmalm GBG, Cofer JB et al. (1990) The influence of preservation injury on rejection in the hepatic transplant recipient. Transplantation 45:103