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Preformed Antibodies in Sensitized Recipients: Effect of Immunoglobulin Titer, Class, and Specificity on Liver and Heart Allografts

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PREFORMED antibodies (Abs) in the blood circulation of recipients before transplantation have been identified as the cause of hyperacute rejection (HAR) of the grafts.[1,2] However, liver allografts are relatively resistant to preformed Abs, and because of conflicting results in clinical practice with sensitized recipients,[3] the practical significance of lymphocytotoxic antibodies (LAbs) in an individual patient is difficult to judge. In this study, we sensitized rats with skin or whole blood and examined in vivo and in vitro characterization of the preformed Abs by actual transplants, LAb assay, flow cytometry, and indirect immunofluorescence (IF) study.

MATERIALS AND METHODS

Animals

Inbred male Lewis ($RT1^l$, LEW) and ACI ($RT1^a$) rats (Harlan Sprague-Dawley, Indianapolis, IN) were used as recipients and donors, respectively.

Sensitization Protocols

LEW recipients were sensitized with either four successive full-thickness ACI tail skin grafts (2 cm diameter) at 14-day intervals or 1 mL heparinized ACI whole blood on two different occasions at a 7-day interval.

Experimental Design

Two to 4 weeks or 12 to 15 weeks after completion of the skin sensitization, or 1 week after the second blood transfusion, the sensitized LEW recipient underwent actual transplantation of ACI heart or liver graft. Hearts were placed in the neck by the method of Heron.[4] Orthotopic liver transplantation was performed according to the method of Kamada.[5] Arterial reconstruction was omitted. Graft rejection was defined by the cessation of the graft heart beat or the death of the animal (liver graft), followed by histological confirmation. Immediately before test transplantation, serum samples were obtained and preformed Abs were characterized by in vitro assays. A complement-dependent LAb assay was performed using unfractionated donor ACI lymphocytes according to the method of Terasaki et al.[6] Titer was defined as the most diluted serum samples with more than 50% cell lysis. For flowcytometry, ACI lymph node cells were incubated with immune LEW serum samples (1:10, 1:100, 1:500, and 1:1000 dilution) followed by fluorescein isothiocyanate-conjugated goat antirat immunoglobulin (Ig)G or IgM Ab (1/75 dilution). For indirect IF study, immune LEW serum was applied to frozen normal ACI liver sections, followed by goat antirat IgM or IgG to detect the target antigen specificity, class, and intensity of binding in the liver.

Statistical Analysis

Results were analyzed by the Mann-Whitney U test. The differences were considered statistically significant if $P < .01$.

RESULTS

Animal Survival

The heart grafts always were hyperacutely rejected in 9 hours when transplanted 2 to 4 weeks after skin sensitization (Table 1). Microscopic examination revealed classic HAR with vascular deposition of IgG. Liver allografts survived longer than heart grafts and were rejected within 3 days (Table 1). Pathologically, marked platelet plugging of the vasculature, congestion, map-like areas of coagulative necrosis, and diffuse vascular deposition of IgG were characteristic findings. Both heart and liver grafts survived significantly longer when transplantation was delayed to 12 to 15 weeks after skin sensitization (Table 1). Pathologically, an accelerated mixed humoral and cellular rejection was nearly as common as pure humoral rejection in failed heart grafts; on the other hand, most of the failed liver grafts showed a mixed humoral and cellular rejection with cellular portal infiltration, platelet plugs, infarcts, and focal sinusoidal IgG deposits. After prior blood transfusions, a slight (statistically insignificant) prolongation of heart allograft and significantly enhanced liver graft survival was seen (Table 1). All heart grafts showed cellular rejection, whereas only one of the failed liver grafts showed classic cellular rejection and the others showed marked sinusoidal Kupffer's cell hypertrophy and sinusoidal lymphohistiocytosis with a modest portal infiltrate.

In Vitro Analysis of the Preformed Antibodies

The highest LAb titer was observed 2 to 4 weeks after the last skin sensitization. Flow cytometry revealed that mixture of strong IgG and relatively weak IgM LAbs were produced. Indirect IF studies revealed intense linear IgG $\geqslant$ IgM reactivity with all vascular endothelial cells, bile

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Table 1. In Vivo and In Vitro Analysis of Preformed Antibodies in Sensitized LEW Recipients

		In Vivo Study							In Vitro Study		
		Heart Graft			Liver Graft						
Sensitization	Time (weeks)	n	Survival (days)	MST	n	Survival (days)	MST	Histology (rejection type)	*LAb Titer (Log_2)	FACS (IgG/IgM)	Indirect IF (IgG/IgM)
None	—	7	6, 6, 6, 6, 6, 7, 7	6	10	9, 9, 9, 9, 10, 10, 10, 11, 12, 13	10	Cellular	—	—	—
Skin	2–4	15	.003, .004, .004, .01, .03, .04, .06, .07, .08, .10, .13, .17, .17, .23, .35	0.07	11	.13, .14, .17, .33, .8, 1.0, 1.0, 1.4, 1.4, 1.7, 3	1.0	Humoral	11.5 ± 1.1	+++/+	+++/++
	12–15	13	.05, .13, .45, .46, .7, .7, .8, 1.0, 3, 3, 4, 4, 5	0.8	12	.9, 3, 3, 4, 6, 8, 8, 9, 9, 9, 9, 11	8	Humoral or mixed (humoral and cellular)	9.3 ± 1.2	++/±	++/–
Blood	1	6	6, 6, 7, 7, 8, 9	7	6	18, 19, 21, 22, 78, 79	21.5	Cellular or no	10.1 ± 1.0	+/++	±/+

MST, median survival time (days).
*Mean ± SD (n = 6–23).

duct epithelial cells, and weaker hepatocyte staining (Table 1). LAb titer was significantly decreased after 12 to 15 weeks from skin sensitization. With flow cytometry, the decrease in titer was noted by a shift to a lower channel intensity for both IgG and IgM. Indirect IF of the sera showed a decreased binding, greater for IgM than IgG compared to the 2- to 4-week sera (Table 1). After blood sensitization, LAb titer was as high as that of 12 to 15 weeks after skin sensitization. Stronger IgM and weaker IgG Ab response compared to those of 2 to 4 weeks after skin sensitization was detected by flowcytometry. By indirect IF, sera after blood sensitization reacted only weakly with hematolymphoid cells (IgM > IgG) amidst the portal connective tissue. The larger vessel endothelium was only weakly or equivocally positive (Table 1).

DISCUSSION

At least three factors—type of organ allograft, time elapsed after priming, and organ used for sensitization—influence allograft outcomes. The liver graft is not only resistant to Ab-mediated rejection[7] but more easily enhanced. And, as others have claimed for cardiac grafts,[8] the time elapsed after priming prolongs the allograft survival with the decrease of LAb titer. This result suggests that determination of Ab titer may increase the predictive value of the cross-match test. However, it is clear that the LAb titer alone does not sufficiently explain the results obtained with different methods of sensitization. Similar high LAb titers were observed in animals that hyperacutely rejected the grafts and experienced enhancement. The better clue to explain such disparate results has come from indirect IF analysis, which showed differences in target antigen specificity in the liver depending upon the method of sensitization. Such an observation is in accord with most clinical studies in which IgG Abs, in contrast to IgM, with endothelial cell specificity have seemed the most dangerous.[9,10] The titer, class, and specificity of Abs varied with the length of time after sensitization or sensitization method. A more precise characterization of preformed Abs may increase the ability to predict outcome of liver transplantation in sensitized recipients or guide pretransplant strategies to foster enhancing Abs.

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