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Complement Activation Correlates With Graft Damage in Baboon-to-Human Liver Xenotransplantation

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COMPLEMENT activation has a critical role in the rejection of vascularized organs transplanted between related and widely separated species.[1,2] In June 1992 and in January 1993 we undertook two baboon-to-human liver xenotransplantations. The serum levels of C3, C4, total hemolytic complement activity (CH_{100}), and circulating immune complexes (IC) were monitored. The objective was to see if there was an association of antibody and complement activation with the rejection of these liver xenografts.

MATERIALS AND METHODS

Two patients, aged 35 and 62 years respectively, both suffered from end-stage liver disease secondary to hepatitis B virus (HBV) infection. Detailed descriptions of the donor and recipient operations and immunosuppression drug doses and their blood levels have been reported elsewhere.[3]

C3 and C4 Levels

C3 and C4 levels were measured by rate nephelometry (Beckman Array, Beckman Instrument Co, Brea, Calif).

Total Hemolytic Complement Activity (CH_{100})

The test is based on the ability of complement to lyse red blood cells. The serum to be tested diffused radially from wells in agarose gel, which contained standardized sheep erythrocytes sensitized with hemolysin. An estimate of CH_{100} was made by comparison of the extent of lysis caused by the test serum sample with that caused by reference sera, run simultaneously. The results were reported in units/mL (normal value > 60 U/mL).

Detection of Circulating Immune Complexes

These complexes were qualitatively detected using zone electrophoresis on agarose gels.[4] In essence, an antibody–antigen immune complex has a net surface charge different from the isolated constituents. This property, together with the clonal restriction of the antibody response, gives rise to distinctive patterns that are apparent in stained agarose gels after routine zone electrophoresis.

RESULTS

Table 1 shows the results obtained from both patients. CH_{100} activity before xenotransplantation was below the limit of detection (<21 U/mL) in both patients, along with abnormally low C3 and C4 levels, which is characteristic of end-stage liver disease. IC were detected before transplantation only in patient 1.

After xenotransplantation, IC were detected from days 1 to 9 in patient 1, and were not detected at any time in patient 2. CH_{100} activity was below the limit of detection during days 1 to 11 in patient 1, and during days 1 and 2 in patient 2. CH_{100} activity in both patients was associated with a reduction in C3 and C4 serum levels. At this time, liver biopsies showed an increase of IgG, IgM antibodies, and complement deposition in hepatic sinusoids in liver biopsy specimens.

Table 1. Circulating Immune Complexes, Total Hemolytic Complement Activity, and C3 and C4 Levels After Baboon-to-Human Liver Xenotransplantation

	Day	IC	CH_{100} (U/mL)	C3 (mg/dL)	C4 (mg/dL)
Patient 1	Pre-Tx	+	<21	35	7
	1	+	<21	33	3
	3	+	<21	27	3
	5	+	<21	27	5
	7	+	<21	33	6
	9	–	<21	40	8
	11	–	21	29	6
	17	–	55	64	17
Patient 2	Pre-Tx	–	<21	43	7
	1	–	<21	30	6
	3	–	36	7	2
	5	–	40	35	6
	7	–	32	34	5
	9	–	53	40	6
	11	–		38	7
	17	–	59	71	8

IC: immune complexes; CH_{100}: Total hemolytic complement activity (NV > 60 U/mL); C3: NV 83–177 mg/dL; C4: NV 15–45 mg/dL.

DISCUSSION

The liver is the main site of complement synthesis.[5,6] The significant reduction of C3 and C4, along with CH_{100} below the limit of detection before transplantation, is known to be secondary to the end-stage liver disease and a reduction in complement synthesis. This could contribute to avoidance of hyperacute rejection in these two baboon-to-human liver xenotransplantations.

Patient 1 had circulating IC before transplantation, which persisted during the first 9 days after transplantation. In contrast, IC were not detected before nor after

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transplantation in patient 2. The correlation between disappearance of circulating IC with detection of CH_{100} activity in patient 1 suggested that the complement produced by the new liver was primarily used to remove circulating IC.[7] Reductions of C3 and C4 at the time of CH_{100} activity have been associated with activation of the classic antibody-dependent complement pathway in experimental models of xenotransplantation between closely related species.[2] One interpretation of our results could be that when complement levels reached a functional level, a humoral reaction occurred in the liver xenograft, reflected by an impairment of the liver function tests and extensive deposits of immunoglobulins (IgG and IgM) and complement (C3 and C4) in the liver biopsy specimens. These deposits largely disappeared 12 days later.[8]

In summary, the complement activation in our baboon-to-human liver xenotransplantations did not result in hyperacute rejection, but we believe that the xenograft was damaged by a mechanism similar to the Schwartzman or local Arthus reactions. New approaches to control complement activation should be included among the therapeutic strategies in baboon-to-human xenotransplantation.

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