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ORIGINAL ARTICLE

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The evaluation of graft irradiation as a method of preventing hemolysis after ABO-mismatched renal transplantation

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Abstract Hemolysis may occur after organ transplantation. In most cases, hemolysis is drug-related, such as to cyclosporin, etc. However, it is important to consider graft-versus-host antibody formation as one of the causes of hemolysis. We evaluated the effect of local graft irradiation as a method of preventing hemolysis arising from ABO antibody formation after ABO-mismatched renal transplantations. The participants in this study were 44 patients who had undergone ABO-mismatched renal transplantation. Of these patients, 23 were subjected to postoperative local irradiation, and 21 were not. We examined the characteristics of anti-blood-type antibodies, and we also compared the frequency of the development of antibody formation and hemolysis development between the groups. The development rates of anti-ABO-antibody formation and hemolysis were significantly higher in the patients without local irradiation (15/21, 71%; 6/21, 29%) than in those with local irradiation (1/23, 4%; 0/23, 0%). The elevated antibodies mainly belonged to the IgG class, not the IgM class. The hemolysis- and antibody formation observed in the patients originally without postoperative local irradiation was dramatically improved by graft irradiation. Local graft irradiation after ABO-mismatched renal transplantations may be needed to prevent the formation of anti-ABO antibodies and to impede the development of hemolysis.

Keywords Graft irradiation · Hemolysis · ABO-mismatched renal transplantation

Introduction

Acquired hemolytic anemia due to anti-A or anti-B antibody formation has been reported to follow ABO-mismatched transplantation [1, 3, 8, 9, 11, 12]. The ABO antibodies arise from ABO-unmatched transplantations, that is, transplantation of a blood-group O organ into a non-O recipient, or of a non-AB organ into an AB recipient. Graft-versus-host antibody reactions by donor passenger B lymphocytes are believed to be the primary mechanism of the hemolysis observed in patients after ABO-mismatched renal transplantations. Several procedures have been proposed to prevent antibody-induced hemolysis; prevention of transplantation of donor lymphocytes by removing lymph nodes from the perirenal fat tissue, etc. [4]. However, there have been few reports on the efficacy of postoperative local irradiation on antibody production. In this study, we analyzed the frequency of antibody production and hemolysis in patients with and without local graft irradiation after ABO-mismatched renal transplantation at our institution.

Patients and methods

Patients

As shown in Table 1, a total of 23 patients (group 1) received grafts from living-related donors with a minor ABO mismatch, between March 1998 and March 2000. In the induction phase, methylprednisolone (MP), cyclosporin (CsA) and azathioprine were given [6]. MP administration was started at a dose of 250 mg/day on the day of transplantation and reduced to a maintenance dose of 8 mg/day within six months, postoperatively. Oral administration of CsA, 6 mg/kg per day, was started 2 days before transplantation, and drip infusion, 2 mg/kg per day, was administered on the day of transplantation. Doses of CsA were administered so as to yield a trough concentration of 200–250 ng/ml within 1 month postoperatively. After that, doses were tapered to maintain a trough level of 150–200 ng/ml. Azathioprine was started 2 days before transplantation at a dose of 1 mg/kg per day orally. Local irradiation of the graft was performed at a dose of 1.5 Gy on days 1, 3, and 5 after transplantation. Between April 1997 and September 2000, a total of 21 patients (group 2) received living-related grafts with a minor ABO mismatch. The patients were maintained on an immunosuppressive protocol similar to the one described above, however, with informed consent from these 21 patients, we did not perform postoperative local irradiation of the graft. Diagnosis of hemolysis was made as follows: (1) positive in the direct anti-globulin test; (2) positive in the crossmatch test; (3) chemical laboratory data such as a drop in hemoglobin and a rise in serum bilirubin; (4) clinical symptom.

The relevance of renal graft weight to antibody production

We report that the frequency and severity of antibody production and hemolysis generally increase with the size of the organ in ABO-mismatched transplants [11]. To study the relevance of renal graft size to antibody production, we routinely measured the renal graft weight at the time of bench surgery during the operation.

Serum anti-A or/and B antibody titers

Antibody titers and serum creatinine were measured postoperatively every day until the patients' discharge. IgM anti-A and B levels were determined by the saline and bromeline agglutination technique, and the indirect Coomb's test was used to measure the IgG titers, as described elsewhere [10]. Patients displaying more than a two-fold increase of postoperative antibody titers over the preoperative titers were classified as producing antibodies.

Statistical analysis

Comparisons between groups 1 and 2 were performed using Student's paired t-test. $P < 0.05$ was considered to be significant.

Results

The frequency of antibody production and development of hemolysis was compared between groups 1 and 2. Antibody production and the development of hemolysis were observed more frequently in group 2 than in group 1. In 15 of 21 patients in group 2 (15/21, 71%), the median anti-blood-type antibody titer was 1:64 by the IgG methods. In the remaining six patients of group 2, the median titer was 1:4 by the IgG methods. By the IgM methods, the titer averaged exactly 1:2 in all patients of group 2. In all patients of group 1, the median antibody titer was 1:4 and 1:2 by the IgG methods and the IgM methods, respectively. The antibody titer of one patient (1/23, 4%) in group 1 was elevated to 1:32, but it decreased to the normal level without any change in immunosuppressive protocol (Table 2). As shown in Fig. 1, IgG antibody was detected between 2 and 6 weeks

Table 1. Clinical data in patients after ABO mismatched renal transplants

Parameter	Group 1: irradiated	Group 2: not irradiated
n	23	21
Gender (M/F)	12/11	11/10
Mean (SD) serum creatinine level at time of observation (mg/dl)	1.5 (0.8)	1.7 (0.4)
Donor-recipient blood type		
0-A	15	9
0-B	4	7
0-AB	1	4
A-AB	2	0
B-AB	1	1
Immunosuppressive drug	CsA, MP, azathioprine	CsA, MP, azathioprine

Table 2. The frequency of antibody production and hemolysis

Parameter	Group 1: irradiated	Group 2: not irradiated		*P*
Antibody production	1/23 (4%)	15/21 (71%)		< 0.01*
		Patients with elevated titer	Patients without elevated titer	–
Mean value of IgG antibody titer	×4	×64	×4	–
Mean value of IgM antibody titer	×2	×2	×2	–
Hemolysis	0/23 (0%)	6/21 (29%)	6/21 (29%)	< 0.01*

*$P < 0.05$ = significant

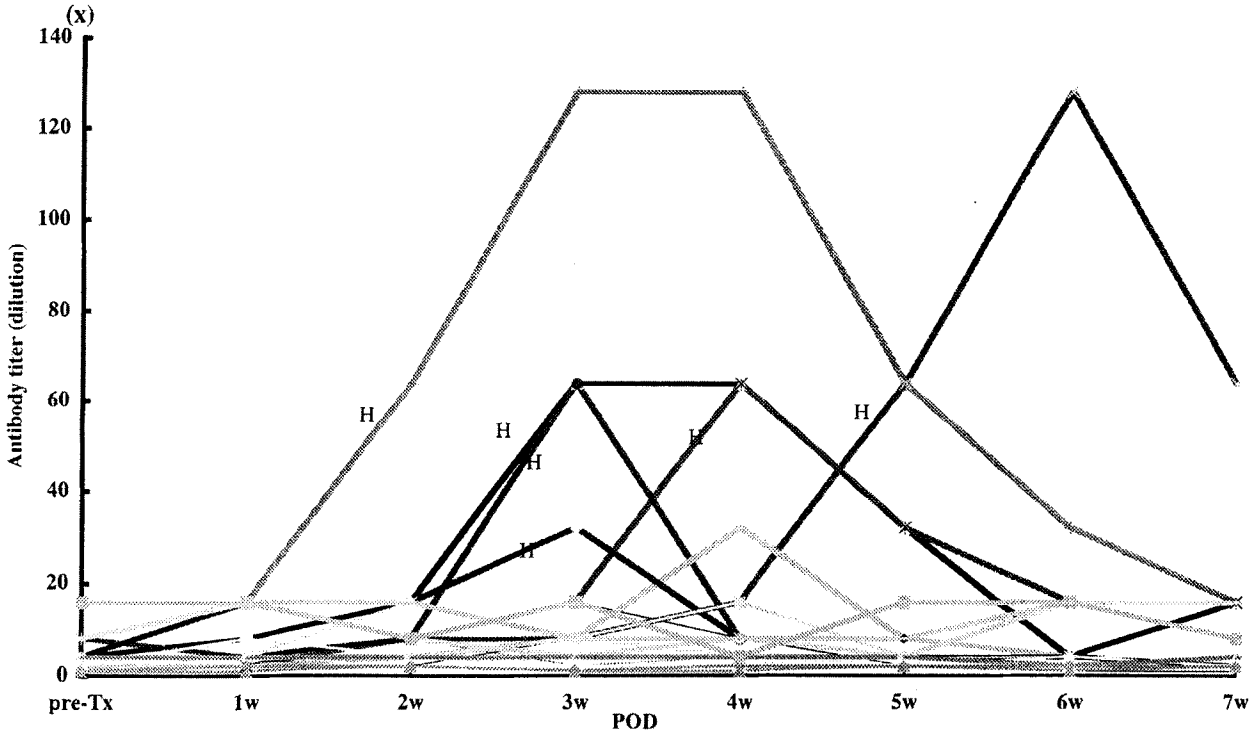

Fig. 1. Changes of IgG antibody in group 2 ($n = 21$). IgG antibody was detected between 2 and 6 weeks postoperatively. Moreover, hemolysis was observed at 20 postoperative days on average. Hemolysis developed before the antibody titer reached the maximum. *H* Time point when hemolysis was observed, *POD* postoperative days

postoperatively. Moreover, hemolysis was observed on average 20 days after operation. Hemolysis developed before the antibody titer reached the maximum.

Hemolysis was observed in six (6/21, 29%) of the group 2 patients, and all six patients showed an elevation of their anti-blood-type antibody titer (Table 2; Fig. 1). One of the patients with hemolysis showed an elevated antibody titer of over 1:128 on day 23 after transplantation, and developed hemolytic anemia with a sudden drop of hemoglobin from 11.0 to 5.4 g/dl. During this period, the serum bilirubin level increased to 2.8 mg/dl, and lactic dehydrogenase rose to 1,323 U/l. The reticulocyte count increased to 55×10^9/l, the serum creatinine level increased from 1.0 to 2.1 mg/dl, and a graft biopsy was performed. Pathological examination of the renal graft demonstrated severe aggregation of cells that had infiltrated the graft, mainly activated B lymphocytes that stained with CD19 and donor specific DR antigen (DR2), suggesting that those cells were of donor origin (data not shown). Because of the gradual deterioration of renal graft function, local irradiation and donor-type blood transfusion were performed without additional anti-rejection therapy, on postoperative day 25. After local irradiation of the cells infiltrating the graft, the clinical symptoms and abnormal laboratory data eventually returned to normal. The other five patients with hemolysis in group 2 also showed an antibody titer increase to 1:64 on postoperative day 20, but the abnormal laboratory data of these patients were milder than those of the previous patient. The abnormal hemoglobin and serum bilirubin levels and the anti-blood-type antibody titer recovered to normal within 2 months without any treatment. Graft function in the patients during this period was excellent. Nine patients with elevated antibody titers in group 2, who did not exhibit hemolysis, required no treatment. The antibody titer levels gradually declined to normal within 6 weeks in all these patients. Only one patient in group 1 with an antibody titer elevated to 1:32 had no evidence of hemolysis among either the clinical or laboratory findings.

Discussion

This study demonstrated that the development rates of anti-blood-type antibody formation and hemolysis were significantly higher in the patients without local irradiation than in the patients with local irradiation after ABO-mismatched renal transplantations. To examine the effect of local irradiation of the graft on antibody production and hemolysis, without the influence of immunosuppressive drugs, we used similar immunosuppressive regimens for all patients in this study, because,

as reported by several researchers [7, 13], the patients receiving CsA or azathioprine have a higher frequency of antibody production and hemolysis than those receiving other immunosuppressive drugs. In this study, the overall rate of antibody formation and hemolysis in the patients receiving CsA and azathioprine was almost the same as in other reports on patients who received immunosuppressive drugs such as mizoribine or OKT3 [7, 12, 13]. This shows that conventional immunosuppressive drug action did not influence secondary B-cell immune responses, such as antibody formation. Future studies of the new macrolide immunosuppressive agent FK506 or mycophenolate mofetil will be of interest in this regard.

The analysis of the effect of donor and recipient blood groups showed no significant difference in the blood groups of the patients who displayed antibody production (O-B, 5; O-A, 6; O-AB, 3; B-AB, 1), although hemolysis was reported to be more common in the group A recipients [11].

Some researchers report that grafts recovering from hemolysis have a poorer survival time than grafts without hemolysis [5]. In this study, however, there were no significant differences in graft function between the hemolysis and non-hemolysis groups, if the grafts completely recovered from hemolysis. However, further examination is needed for a long follow-up.

It is noteworthy that the average weight of the renal grafts of 15 patients with antibody formation in group 2 was significantly higher than that of the remaining six patients without antibody formation in the same group. Possibly, more passenger B cells from the donor, which are thought to produce anti-blood-type antibodies, remain in the graft at the time of transplantation. One patient with antibody elevation in group 1 also received a larger graft (224 g). This finding suggests that in larger grafts thorough flushing to wash out passenger lymphocytes may be necessary to prevent antibody formation or hemolysis, unless local graft irradiation is scheduled. In general, ABO antibodies are detected as a result of a secondary immune response by previously primed B lymphocytes within a few days of transplantation, and they disappear within 2 months [2]. On average, six patients in group 2 developed hemolysis on postoperative day 20. The hemolysis was accompanied by elevation of the anti-blood-type antibody titer, mainly IgG. To prevent lethal complications of severe hemolysis by removal of anti-blood-type antibodies, regular screening for anti-blood-type antibodies is necessary for a short time after ABO minor mismatched renal transplantations.

In conclusion, we found a higher rate of antibody formation and development of hemolysis in the patients without irradiation after ABO-mismatched renal transplantations than in the patients with irradiation. We conclude that local irradiation of the graft may play an essential role in inhibiting the secondary B-cell response that causes antibody formation in patients after ABO minor mismatched renal transplantations. Also, new immunosuppressive regimens such as mycophenolate mofetil to suppress B-cell activity are anticipated to avoid ABO-antibody-mediated hemolysis after transplantations across blood-type barriers.

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