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Development of Alloantibodies to Non-HLA and HLA Systems in Patients on Transfusion Therapy

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Abstract Nineteen patients with aplastic anemia or hematological malignancies were prospectively examined with regard to the development of alloantibodies following blood transfusions. Sera obtained at regular intervals were serologically analyzed to detect the lymphocytotoxic antibodies against T cells, B cells and monocytes from a panel of unrelated subjects or their HLA-identical siblings, and the following results were obtained. Anti-HLA-A,B,C, and DR antibodies were detected in 9 out of the 19 patients. Among 9 patients who received the preceding transfusions, three had the preformed antibodies. One patient who underwent the preceding pregnancies was already sensitized. Five out of the 15 nonsensitized patients developed transfusion-induced alloantibodies during the period of this study. Three out of the 19 patients demonstrated B cell specific antibodies. The development of anti-HLA-DR antibodies and B cell specific antibodies prior to the occurrence of anti-HLA-A,B, and C antibodies was observed in 3 cases. The development of monocyte specific antibodies was not observed in this study. The development of these alloantibodies was not dependent on the total volume of blood components transfused. From these results, it is concluded that blood transfusions to the selected candidates for bone marrow transplantation should be reduced to the minimum unless indicated by urgent medical necessity.

Key Words: Alloantibody, Non-HLA antigen, HLA antigen, Blood transfusion

Introduction

Bone marrow transplantation (BMT) has been used with increasing success for the treatment of patients with severe aplastic anemia and hematological malignancies. However, graft rejection is still the most prominent cause of failure. Clinical result in untransfused patients (1) and experimental data in dogs (2) strongly suggest that graft rejection is largely due to sensitization of the recipient by blood transfusions to non-HLA antigens. The development of monocyte specific antibodies in recipients of HLA-identical marrow transplantation has been demonstrated and their occurrence in combination with the presence of antibody-dependent cell-mediated cytotoxicity (ADCC) is shown to correlated with a high incidence of graft rejection (3). In addition, a signifi-

cant relationship between the development of monocyte specific antibodies and graft rejection is also pointed out by Gluckman et al. (4).

Sensitization against B cell specific alloantigens other than DR locus has also been reported by Maeda and Juji (5). However, the role of B cell specific antibodies in the outcome of BMT still remains to be evaluated.

To date, most of investigational interest in this area has been concerned with the effect of preceding transfusion on graft outcome, and the natural history of sensitization to HLA and non-HLA antigens induced by transfusion is not adequately studied. The present study was designed to investigate prospectively the patients with aplastic anemia and hematological malignancies and to assess the incidence of alloimmunization by blood transfusion against non-HLA and HLA antigens.

Materials and Methods

Nineteen patients with different causes of hematological abnormalities such as leukemias, lymphomas, myelofibrosis, or aplastic anemia were prospectively studied over a period ranging from 2 to 9 months. Fourteen patients were male and 5 were female. The age of patients ranged from 13 to 66 years. The clinical data of these patients are shown in Table 1. All patients with hematological malig-

Table 1 Clinical Data of 19 Patients with Bone Marrow Failure

Patient	Age	Sex	Diagnosis*	Period of Observation	Treatment**	Comments
1	26	F	AML	7	Chemo.	died
2	24	M	AML	3	Chemo.	alive
3	66	M	AML	3	Chemo.	died
4	45	M	APL	3	Chemo.	alive
5	39	M	ALL	8	Chemo.	died
6	46	M	ALL	2	Chemo.	died
7	19	M	MKL	5	Chemo.	died
8	34	M	MKL	4	Chemo.	died
9	34	M	CGL-BC	2	Chemo.	died
10	37	M	MF	4	An.	alive
11	53	M	NHL	3	Chemo.	auto-BMT, died
12	52	M	NHL	9	Chemo.	auto-BMT, died
13	62	F	NHL	3	Chemo.	died
14	13	M	BL	6	Chemo.	died
15	25	M	APA	5	An.+Gl.	alive
16	18	M	APA	2	An.	alive
17	43	F	APA	3	An.+Gl.	alive
18	35	F	APA	3	An.+Gl.	allo-BMT, died
19	20	F	APA	3	(−)	allo-BMT, alive

* AML=acute myeloblastic leukemia APL=acute promyelocytic leukemia
ALL=acute lymphoblastic leukemia MKL=acute megakaryoblastic leukemia
CGL-BC=chronic granulocytic leukemia in a blastic crisis
MF=myelofibrosis NHL=non-Hodgkin lymphoma
BL=Burkitt's lymphoma APA=aplastic anemia
**Chemo.=chemotherapy An.=androgens Gl.=glucocorticoids

nancies were treated with cytostatic drugs. Serum samples were collected at regular intervals. Prior to this study, 10 out of the 19 patients had never recieved the preceding transfusion of any blood components.

The sera were inactivated at 50°C for 30 min and stored at −40°C until use. Heat-inactivated sera from healthy random, non-immunized blood-group-AB subjects were used as a negative control A polyspecific serum that contained anti-HLA antibodies was used as a positive control.

Twenty milliliters of heparinized peripheral blood were collected from HLA-A,B,C, and DR typed healthy selected donors. The HLA antigens of these blood cells included A 2, 11, w24, w31, B7, w35, w39, w46, w51, w52, w54, w56, w61, w62, Cw1, w3, w7, X46(6), DR1, 2, 4, 8W6Y, NJ2(6), w8, w9. T cells were tested by NIH standard technique (7). B cells were isolated by the thrombin-nylon wool method described by Danilovs et al. (8) and incubated with antisera for 1h at 37°C followed by incubation with rabbit complement for 2h at 22°C. Monocytes were separated using the glass petri dish method described by Satake et al. (9) and incubated with antisera for 3h at 37°C and for 2h at 22°C following the addition of rabbit complement.

Results

The sera of 19 patients were examined at regular intervals to detect alloantibodies (Table 2). A serum was considered to contain antibodies when it repeatedly gave a positive reaction with at least one of the panel cells. Nine out of the 10 untranfused

Table 2 Transfusions and Development of Alloantibodies

Patient	Preceding Transfusions	Preformed Antibody	Development of Antibody during Study	Total Units of Transfusions				Units of Transfusions until Antibody Formation			
				RBC	MDP	SDP	WBC	RBC	MDP	SDP	WBC
1	+	−	−	171	1828	0	37				
2	+	−	−	29	319	0	0				
3	−	−	+	100	398	1	0	17	89	0	0
4	−	−	+	27	253	0	10	12	50	0	0
5	+	−	+	17	70	0	0	14	56	0	0
6	−	−	−	16	60	0	1				
7	−	−	−	16	121	0	0				
8	+	+		35	178	0	5	12	7	0	0
9	−	−	−	9	10	0	0				
10	+	+		75	243	1	0	5	0	0	0
11	−	−		59	359	0	1				
12	−	−	−	70	467	1	0				
13	−	+		6	0	0	0				
14	−	−	−	14	87	0	0				
15	+	−	−	43	82	0	0				
16	+	−	−	36	120	0	0				
17	+	+		11	22	0	0	5	0	0	0
18	+	−	+	42	0	8	0	16	0	0	0
19	−	−	+	11	0	7	0	9	0	0	0

RBC=leukocyte-poor red cells
MDP=multiple-donor platelet concentrates
SDP=single-donor platelet transfusion
WBC=single-donor granulocyte transfusion

patients gave negative lymphocytotoxicity tests with T cells, B cells and monocytes of panel donors at the beginning of this study. One patient who had prior pregnancies gave positive lymphocytotoxicity tests.

Among 9 patients who had recieved the preceding blood transfusions, three showed a positive lymphocytotoxicity. Five patients developed lymphocytotoxic antibodies during this study. Analysis of combined reactions against T cells, B cells and monocytes demonstrated three distinct patterns of reactivity (Table 3). Sera from 5 immunized patients reacted with T cells, B cells and monocytes from several panel members, and they were referred to as [T+B+M+], which indicated the presence of HLA-A,B, and C antibodies in these sera. Sera of all these immunized patients reacted with B cells and monocytes but not with T cells from other donors. This reactive pattern of [T−B+M+] suggested the presence of HLA-DR antibodies. In 3 out of above 5 patients, a positive cytotoxicity was noted only against B cells from different donors, showing the reactive pattern of [T−B+M−]. Monocyte specific reaction pattern [T−B−M+] was not demonstrated in this study. No further analysis have yet been done to define the nature of the antibodies.

Patients who had no antibody at the beginning of this study

As shown in Table 2, 5 (Case 3, 4, 5, 18, 19) out of the 15 patients (33%) who had no antibody at the beginning of this investigation developed lymphocytotoxic antibodies during this observation period.

As seen in Table 3, two (Case 3, 19) out of the 5 immunized patients demonstrated [T−B+M+] pattern prior to the development of [T+B+M+] pattern, indicating that HLA-DR antibodies were produced earlier than HLA-A, B, C antibodies in these patients. One patient (Case 5) showed only [T−B+M+] pattern. Two patients (Case 4, 18) demonstrated [T+B+M+], [T−B+M+] and [T−B+M−] patterns during the period of this study.

Two patients (Case 18, 19) with aplastic anemia were treated with BMT from their HLA-identical siblings. One patient (Case 18) died from interstitial pneumonitis on the 15th day after BMT and the evaluation of graft rejection was impossible. Another patient (Case 19) had engraftment. Both of them

Table 3 The nature of lymphocytotoxic reactivity in patients with alloantibodies

Patient	Patterns of Reactivity					
	Initial Stage			Final Stage		
	[T+B+M+]	[T−B+M+]	[T−B+M−]	[T+B+M+]	[T−B+M+]	[T−B+M−]
3		+		+	+	
4	+	+	+	+	+	+
5		+			+	
8		+			disappeared	
10		+		+	+	
13		+	+		disappeared	
17		+		+	+	
18*	+	+	+	+	+	+
19*		+		+	+	

* Data before bone marrow transplantation

had already developed antibodies directed to T cells, B cells and monocytes before BMT, but crossmatches of their sera with T cells, B cells and monocytes from their HLA-identical siblings were negative. The alloantibodies of one patient (Case 19) disappeared after BMT but after 60 days of BMT antibody of [T−B+M+] pattern developed again. It was suggested that the production of antibodies by engrafted donor cells occurred aganist transfused blood cells.

The relationship between the formation of alloantibodies and the number of units of blood cells administered was investigated (Table 2). In patients with hematological malignancies who developed alloantibodies, the mean amounts of transfusions given before the development of alloantibodies were 12 units of leukocyte-poor red cells (RBC) and 40.4 units of multiple-donor platelet concentrates (MDP). Non-immunized patients with hematological malignancies who had no history of transfusion before this study recieved an average of 30 units of RBC, 184 units of MDP, 0.2 units of single-donor platelet transfusion (SDP) and 0.3 units of single-donor granulocyte transfusion (WBC). Immunized patients with aplastic anemia recieved an average of only 10 units of RBC prior to the development of alloantibodies. Interestingly non-immunized patients recieved more transfusions than immunized ones.

Two out of the 4 patients with aplastic anemia were female and both of them developed alloantibodies. Two male patients with aplastic anemia were not immunized during this observation.

In patients with hematological malignancies, the average age of immunized patients was 50 years and that of non-immunized ones was 33 years. In patients with aplastic anemia, the average age of immunized patients was 30 years and that of non-immunized ones was 33 years.

Although there was a tendency that elder patients with hematological malignancies were easily sensitized, the number of patients examined seems to be too small to evaluate the effect of age on the development of alloantibodies.

Most of the patients with hematological malignancies developed alloantibodies within 2 months after the first transfusion. Two patients (Case 18, 19) with aplastic anemia developed alloantibodies within a month. One patient (Case 18) had the history of pregnancies and developed alloantibodies within 2 weeks.

Patients who had preformed antibodies at the beginnig of this study

Three of the 4 patients who had preformed antibodies demonstrated [T−B+M+] pattern at the beginning of this study. One of these 4 patients showed both [T−B+M+] and [T+B+M+] patterns. Two patients developed [T+B+M+] pattern during this study, but the antibodies of the other patients disappeared after the treatment with cytostatic drugs.

Discussion

Although BMT from an HLA-identical sibling is an effective form of therapy for the patients with aplastic anemia and hematological malignancies, graft rejection is the most serious problem, which is difficult to predict. The influence of sensitization to non-HLA antigens on the fate of grafts has been suggested in kidney and bone marrow transplantations even when the major histocompatibility complex is identical between donor and recipient. It is likely that graft rejection is, for the most part, attributable to sensitization of the recipient to non-HLA systems induced by blood transfusions. Lymphocytotoxic antibodies are often found in the sera of the patients with bone marrow failure (10) (11). These antibodies are directed not only to HLA gene products, but also to other antigens which are expressed only on lymphocyte subpopulations. This study

was aimed to investigate prospectively the patients with aplastic anemia or hematological malignancies with regard to the development of alloantibodies against both HLA and non-HLA antigens induced by transfusions and to evaluate the nature of antibodies in these patients. In this study, reactions to T cells, B cells, and monocytes were simultaneously tested. HLA-A, B, and C antigens are distributed on T cells, B cells, monocytes, granulocytes, and platelets, and sera which contain anti-HLA-A,B, and C antibodies are referred to as [T+B+M+]. Gluckman et al. (12) reported non-HLA lymphocytotoxins directed to T cells and B cells detected in the sera of transfused patients. In order to determine whether the antibodies which show the reactive pattern of [T+B+M+] are anti-HLA antibodies or not, absorption study with platelets and family segregation study are necessary. In this investigation, these studies to define the nature of the antibodies have not been done yet.

Six of the 19 (32%) patients showed the reactive pattern of [T+B+M+]. Dutcher et al. (13) reported that the frequency of alloimmunization among patients with acute nonlymphocytic leukemia recieving frequent platelet transfusions was 38 per cent. In their study, serum samples were tested with whole cells containing T cells, B cells, and monocytes, and it was difficult to detect the antibodies reacting lymphocyte subpopulations. Pagels et al. (14) reported that 30% of the patients with bone marrow failure of various causes developed anti-HLA-antibodies. As concerned with HLA-A,B, and C antibodies, the immunization rate of our series was essentially similar to that of reported previously.

According to Storb et al. (11), the presence of lymphocytotoxins detected by the tests using whole cells does not predict marrow graft rejection. Gluckman et al. (12) reported that non-HLA lymphocytotoxins directed to T cells and B cells did not correlate with graft rejection in BMT.

In this study, one patient (Case 19) developed antibodies of [T+B+M+] pattern, but graft rejection was not observed.

HLA-DR antigens are distributed on B cells and monocytes. Sera which contain anti-HLA-DR antibody show the reactive pattern of [T−B+M+]. All immunized patients, 10 out of the 19 patients (53%), revealed this pattern. It is reported that in kidney transplantation B cell positive crossmatches with anti-HLA-DR antibodies show deleterious effects on the graft outcome (15). In BMT, the major histocompatibility complex must be identical between donor and recipient. The influence of anti-HLA-DR antibodies on the graft outcome in BMT is not known yet.

Although antibodies against HLA-A,B,C and DR antigens were the most frequently formed alloantibodies, B cell specific alloantibodies were also found in 3 (Case 4, 13, 18) of the 19 patients (16%). In 4 cases (Case 3, 10, 17, 18), the development of antibodies against HLA-DR antigens and B cell specific antigens was observed prior to the development of those against HLA-A,B, and C antigens. B cell specific alloantigen system, which is segregated with HLA-antigens and is coded for in the HLA-D/DR region, is demonstrated using antisera obtained from multiparous women. The role of this system in the outcome of bone marrow and kidney transplantations is not fully understood.

Analizing the sera of transfused patients, Gluckman et al. (4) demonstrated non-HLA antibodies which were directed only to B cells. They reported that these antibodies had no significant effect on the graft outcome in BMT.

The development of monocyte specific antibodies was not noted prior to that of anti-HLA antibodies. Anti-HLA antibodies show the reactive pattern of [T+B+M+] or [T−B+M+], and [T−B−M+] pattern is masked

when examined by the standard method. Therefore, in order to detect the monocyte specific antibodies in the patients who have the preformed anti-HLA antibodies, it is necessary to absorb anti-HLA antibodies or to crossmatch the sera with monocyte from their HLA-identical siblings. In this study, crossmatches of sera of two patients, who recieved BMT from their HLA-identical siblings, with T cells, B cells, and monocytes from donors were negative. One patient showed engraftment and the other was not evaluable for graft rejection because of early death from interstitial pneumonitis. It is reported that the development of monocyte specific antibodies in recipients of HLA-identical BMT in combination with the presence of ADCC correlates with a higher incidence of graft rejection (3). According to Gluckman et al. (4), there is a significant relationship between graft rejection and the development of monocyte specific antibodies.

The present results indicated that the development of alloantibodies is not dependent on the total volume of blood components transfused but on the immune responsiveness of an individual patient. These data are in agreement with the result of Dutcher and coworkers (13) who reported that transfusion-induced immunization was not dose-dependent. A rapid development of alloantibody in patients with aplastic anemia compared with that in patients with hematological malignancies might be attributable to the absence of chemotherapy which has a potent immunosuppressive effect.

As for the effects of sex and age on the occurrence of alloantibodies, it is difficult to make a conclusion because of small series of this study, but young female patients with aplastic anemia seemed to be most easily sensitized. Dutcher et al. (13) reported that a rapid development of alloantibodies was noted in some female patients who had been pregnant previously. They ascribed this phenomenon to the anamnestic response. In this study, one patient (Case 18) who had a history of pregnancies showed the development of alloantibodies within two weeks following the initial blood transfusion.

In summary, this prospective study revealed the development of alloantibodies against HLA and non-HLA antigens in about 50% of the patients who recieved blood transfusions. At present, it is difficult to predict the sensitization associated with transfusions. Therefore, transfusions of granulocytes or platelets to the selected candidates for BMT should be reduced to the minimal requirement and leukocyte-poor red cells should be used for the correction of anemia.

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