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Evaluation of Graft-Versus-Host Disease Based on Measurement of HLA Levels in the Plasma of Allogeneic Bone Marrow Recipients

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HLA levels were estimated in the plasma of allogeneic bone marrow recipients with and without graft-versus-host disease (GVHD). It was found that the level of plasma-soluble HLA is elevated in recipients with developing acute GVHD and that a rise in these antigens coincides with the onset of clinically manifest GVHD.

Key Words: *inhibition of anti-HLA antibodies; acute (chronic) graft-versus-host disease; plasma-soluble HLA*

The graft-versus-host reaction, or graft-versus-host disease (GVHD), which is also known as secondary disease, is a grave and not infrequently fatal complication of an allogeneic bone marrow transplant [3,4, 6,8]. Research on this immunological complication is of great importance to those working in the transplantation and blood transfusion field.

Laboratory criteria for evaluating GVHD have been insufficiently developed. The reported elevation of HLA antigens in the plasma of allogeneic bone marrow recipients developing GVHD [11] prompted us to undertake the study described below.

MATERIALS AND METHODS

Sera from 20 allogeneic bone marrow recipients with GVHD of varying severity or free of this disease were examined. The recipients included 13 men and 7 women aged 15 to 39 years (mean age 21 years), 13 of whom had acute leukemia, 6 had chronic myelocytic leukemia, and 1 had lymphosarcoma. Ten patients had been prepared for the bone marrow transplant by being given cyclophosphamide in two doses (total dose=120 mg/kg body weight), followed by total-body irradiation at 2 Gy twice daily for 3 days (total dose 12 Gy) [4], while the other 10 had received Myelosan for 4 days (total dose 16 mg/kg body weight) and cyclophosphamide as indicated above. Bone marrow donors for all 20 patients were sibs identical for both parental HLA haplotypes [1] which did not react in the mixed lymphocyte culture. For the prevention of GVHD, the patients had been given methotrexate and cyclosporine A [9]. Five of them exhibited no signs of GVHD over 57 months of the post-transplant follow-up period.

Acute GVHD was clinically diagnosed in 8 recipients 19-50 days postgraft (34.5 days on average) and defined, in accordance with the international

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TABLE 1. Clinical Evaluation of Organ Involvement in Eight Marrow Transplant Recipients with Acute GVHD

Recipient	Diagnosis	Stage of acute GVHD	Extent of organ involvement, scores (+)		
			skin	liver	gastrointestinal tract
V.	Acute myelomonoblastic leukemia	I	+	-	-
G.	Acute myeloblastic leukemia	I	+	-	-
K.	Acute myeloblastic leukemia	I-II	+	++	++
R.	Chronic myelocytic leukemia	II	++++	++	-
K.	Chronic myelocytic leukemia	IV	++	++	++++
M.	Acute lymphoblastic leukemia	IV	++++	++++	++++
D.	Chronic myelocytic leukemia	IV	++++	++++	++++
S.	Acute myeloblastic leukemia	III	++	+++	-

classification [2,5], as being stage I-II in 4, stage III in 1, and stage IV in 3, based on the extent of skin, liver, and gastrointestinal tract involvement (Table 1).

Chronic GVHD developed in 7 recipients 3-7 months postgraft (5 months on average), having evolved from acute GVHD in one of them. Six recipients were diagnosed as having limited chronic GVHD. The skin and the liver were affected in 2 patients and the gastrointestinal tract in 3, while one patient developed progressive pancytopenia after a period of stably restored hematopoiesis. Two patients had generalized chronic GVHD featuring lung tissue fibrosis, scleroderma, and a contracture syndrome in addition to extensive skin depigmentation, erythema, and pronounced liver dysfunction. These patients were all administered a course of prednisolone and cyclosporine A therapy; 3 of them had to be put on azathioprine as well.

The principal objective of our immunological examination was to estimate the level of soluble HLA antigens in the plasma of bone marrow transplant recipients during the period of clinically manifest GVHD. The method we used is based on the depletion of alloimmune anti-HLA antibodies with antigens dissolved in the plasma. To this end, a serum containing antibodies to the HLA A or B locus (test serum) was diluted at 1:2, 1:4, 1:8 ... ratios with the recipient's serum taken during clinical manifestations of the graft-versus-host reaction. In the control assays, the test serum was diluted with the recipient's serum taken before the bone marrow transplant and also with the serum from a healthy subject with the AB(IV) blood group. The procedure of anti-HLA antibody depletion was carried out in flat-bottomed 96-well plates for 60 min at room temperature. Thereafter, 1 μl of the contents from each well was transferred to Tarasaki's plates under Vaseline oil and a lymphocytotoxicity test was performed with lymphocytes from a subject sharing one or two HLA antigens with the recipient. The lymphocytes were isolated from defibrinated or heparinized blood and centrifuged in a Ficoll-Verografin gradient having a specific density of 1076-1078 g/cm^3. A reproducible decrease of the antibody titer by one dilution or more in the test serum

TABLE 2. Titers of Anti-HLA Antibodies in the Test Serum before and after Its Depletion with Sera from Bone Marrow Transplant Recipients with Different Forms of GVHD

Recipient	Form and stage of GVHD	Time when clinical signs of GVHD appeared	Time when plasma-soluble antigens were detected	Titer of anti-HLA antibodies	
		days after transplantation		before depletion	after depletion
K.	Acute, IV	37	40	1:256	1:8
V.	Acute, I	21	23-36	1:32	1:8
	Chronic, limited	149	-	1:32	1:32
R.	Acute, II	27	29-37	1:8	1:4
	Chronic, generalized (evolved from the acute form)	153	-	1:8	1:8
I.	Chronic, limited	93	31-123	1:64	1:32
D.	Acute, IV	50	56	1:8	-
S.	Acute, III	37	40	1:8	-

indicated an elevated level of plasma-soluble HLA in the recipient's serum and was interpreted as an indicator of the immunological response in GVHD.

RESULTS

Sera from 20 bone marrow transplant recipients, including 15 with acute or chronic GVHD, were examined. The activity of anti-HLA antibodies in the test serum was found to be markedly reduced after its exposure to the serum from 7 recipients, 6 of whom had acute and one had chronic GVHD (Table 2).

The greatest elevation in the level of plasma-soluble HLA occurred in the recipients with acute GVHD of stage III or IV. In patient K., the acute GVHD diagnosed on day 20 progressed to stage IV by day 34; on day 40, plasma-soluble HLA were present in a high concentration in the blood, as indicated by a sharp fall in the anti-HLA antibody titer of the test serum (from a 1:256 to a 1:8 dilution). Patient D., developed stage IV acute GVHD by day 50 postgraft, after which time plasma-soluble antigens were present at elevated levels in the blood: the test serum with an anti-HLA antibody titer of 1:8 completely lost its activity after exposure to the serum from this recipient. Female patient S., with a diagnosis of stage III acute GVHD on day 37 postgraft, exhibited plasma-soluble antigens in the blood after day 40, for the test serum was totally inhibited by her serum. The elevations of plasma-soluble HLA in the sera from recipients with stage I or II acute GVHD were less marked. The serum from patient V., with stage I acute GVHD inhibited anti-HLA antibodies in the test serum from day 21 to day 36 postgraft, the antibody titer decreasing from 1:32 to 1:8 after exposure to his serum. Patient R., diagnosed as having stage II acute GVHD on day 27 postgraft, had elevated levels of plasma-soluble antigens from day 29 to day 37 (the anti-HLA antibody titer in the test serum decreased from 1:8 to 1:4). The acute GVHD in this patient evolved into the chronic variety, after which no increase in plasma-soluble antigens was recorded. In recipients with chronic GVHD the level of plasma-soluble antigens was not observed to rise. It was only in one patient, I., that the test serum activity decreased (by one dilution) during the period when the first clinical manifestations of chronic GVHD appeared. In no case was a rise in the level of plasma-soluble antigens recorded during the period when the chronic disease was well established. Control assays showed that sera of bone marrow recipients were incapable of depleting the test serum of anti-HLA antibodies before transplantation. Nor did the sera from 5 recipients without clinically manifest GVHD or those from recipients of autologous or syngeneic bone marrow deplete the test serum of these antibodies.

Thus, as is evident from the findings, the sera of allogeneic bone marrow recipients with stage IV acute GVHD acquired a capacity to inhibit the activity of anti-HLA antibodies in the test serum, thereby reducing its titer. The sera from recipients with chronic GVHD was less inhibitory. Immunological signs of GVHD appeared during the development of clinical symptoms of acute disease.

This study indicates that severe forms of acute GVHD involve extensive damage to the recipient's cells, and primarily the cell membranes, with a resultant release of class I HLA into the circulation, as is evidenced by the reduced titers of anti-HLA antibodies in the test serum. Class I HLA of donor origin have also been reported to appear in the blood of allogeneic liver transplant recipients [7]. Inhibition of anti-HLA antibodies may find application as a method of confirming the presence of GVHD and of evaluating its severity.

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