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INTRODUCTION

One of the main goals in histocompatibility research ought to be the identification of non-HLA loci important in graft-versus-host disease (GvHD) and graft rejection. Even if this were achieved, it would only help the clinician identify those patients with HLA-identical siblings who have a minimal risk of GvHD or graft rejection. Although an important contribution, it would not solve the problem of what to do for those patients who do not have an HLA-identical sibling donor who is also identical at these non-HLA loci. In fact, with increasing selectivity based on better typing, the number of these problem patients increases. For such patients who have no related donor, help could come from two directions:

1. The use of unrelated donors compatible for HLA and non-HLA loci. However, we need to know whether matching for HLA between unrelated donor-recipient pairs can reach the same degree of compatibility as that between siblings.
2. More effective immunosuppression.

WHAT PROGRESS HAS BEEN MADE IN RECOGNIZING NON-HLA ANTIGENS?

Successful platelet transfusion holds a key position in bone marrow transplantation, especially in sustaining the patient during and after bone marrow transplantation. In hyperimmunized patients, only about 60 percent of the platelet transfusions will be successful if HLA-matched platelets are used (3).

The Seattle bone marrow transplant group has shown that GvHD is more serious in patients who are resistant to platelet transfusions (26). The possibility of matching for platelet antigens and preventing immunization to them are thus important goals in bone marrow transplantation and will be discussed first. Following the lead of the Seattle group, several studies have shown that the prognosis of bone marrow transplantation, especially as determined by GvHD, is poorer when the donor is a female and the recipient a male (Table 20-1) (1,11,26). The question one is confronted with is whether this is due to an *in vitro* detectable immunity against the H-Y antigen, as has been described in rodents. This question is especially relevant because not all sex-mismatched grafts fail because of GvHD, and such an *in vitro* test would make it possible to identify which donor-recipient pairs were at risk for GvHD.

TABLE 20-1 Successful Bone Marrow Transplantation in Males

DONOR/RECIPIENT	FRACTION OF SURVIVORS		
	Seattle series[a]	E B M T series[b]	S C I D[c] International Bone Marrow Transplant Registry Report[d]
M → M	8/9	17/24	15/32
F → M	7/18	5/21	5/25
p Value	0.02	0.002	0.03

[a]Storb et al. (26).
[b]European Bone Marrow Transplant (EBMT) Cooperative Group (11)
[c]Severe combined immunodeficiency disease.
[d]JAMA (1).

PLATELET ANTIGENS

Brand et al. have recently shown that a near 100 percent success rate can be achieved if HLA matching is supplemented by a sensitive platelet cross-match by an immunofluorescence technique (3). With this technique, a set of non-HLA antigens can be recognized.

It can be anticipated, however, that the incidence of hyperimmunized patients will diminish. Eernisse has shown that sensitization by platelet transfusion can be prevented in over 70 percent of the patients by the use of platelets that have been centrifuged three times and that contain virtually no lymphocytes (8). Eernisse's contribution is thus of direct importance in clinical bone marrow transplantation because of the already-mentioned fact that GvHD is more severe in platelet-resistant patients (26).

H-Y IMMUNITY

Goulmy et al. studied six hyperimmunized women for evidence of anti-H-Y immunity using the cell-mediated lympholyses (CML) test (16). They found significant evidence for such immunity in two patients, both of whom were suffering from aplastic anemia and one of whom had received a bone marrow transplant. It is of interest that this immunity is only evident when both the female effector cells and the male target cells carry HLA-A2 (Table 20-2). It remains to be determined whether this test really predicts H-Y dependent GvHD. Nevertheless, its relevance in allograft reactions is emphasized by the finding that male donor-female recipient kidney grafts that share HLA-A2 have a 2-year graft survival of 38 percent, whereas if they both lack HLA-A2, it is 58 percent (15).

The HLA-A2 restricted H-Y immunity is the first example in man of the dual recognition phenomen that has for some time been recognized in the mouse (33). In mouse systems, the restriction

TABLE 20-2 Sex-related Killing in CML of HLA-/ itive Target Cells

	KILL (PERCENT)	SEX OF HL/ POSITIVE TA
		M
CML	> 30	15
by Mrs. R.'s	10–20	0
effector cells[a]	< 10	0
CML	> 30	6
by Mrs. K.'s	10–20	0
effector cells[a]	< 10	0

[a]The effector cells were HLA-A2 positive.

phenomenon has been described not only fc but also for microbial and artificial antigens The precise mechanism of the dual recognitic nomenon is not known.

The HLA-A2 restricted H-Y immunity demonstrated for more than a year after ir zation, but it eventually wanes. It can, ho be reactivated *in vitro* by stimulation with un HLA-A, HLA-B, and HLA-C identical, but D different lymphocytes.

The serum from one of these patients (M was also examined for serological activity H-Y. The standard, two-step complement-d ent cytotoxicity (CdC) technique was negati van Leeuwen was able to show that a tw fluorescence technique, which enabled the nition of CdC directed to identifiable sub *mononuclear cells showed antibodies that* with HLA-A2 male donors (Table 20-3) (23

TABLE 20-3 HLA-A2 Restricted Anti-H-Y Immu Detected by Serology

TWO-COLOR FLUORESCENCE CYTOTOXICITY TEST	CELL-MEDIATE LYMPHOLYSIS	
	Positive	Neg
+	8	
−	0	1

a fraction of the mononuclear cells that were not typical B-cells were killed.

The interpretation of these two sets of data is difficult. In the case of the CML reaction, one can conceive of two hypotheses. In the first, the killer cell carries two receptors, one for HLA-A2 and one for H-Y. In the second, the killer cell carries one receptor directed to a neoantigen generated in an interaction between HLA-A2 and H-Y.

The serological reactions must be considered separately. Here, there are three possibilities. First, we would be dealing with two antibodies produced by two clones of B-cells directed to HLA-A2 and H-Y; second, we could be dealing with an antibody directed to an neoantigen produced by HLA-A2 and H-Y; and third, it could be that all three antibodies, HLA-A2, H-Y, and the antibody raised by the neoantigen, reacting together are needed for killing to occur. It should be possible to determine which of these situations exists.

It can be said that progress is being made in two areas that are relevant to bone marrow transplant survival. These are platelet alloantigens and immunity to sex-linked transplantation antigens. There are, of course, many more non-HLA systems. The compatibility of some of these systems, such as the ABO blood group system and the tissue system group Five, have been shown not to be relevant to bone marrow transplantation (4,21). For others, such as the granulocyte systems (NA, NB, 14) and the endothelial and monocyte system E, relevancy has not been tested for (18,19,24). For none of these systems, with the exception of the ABO system, has it been ascertained by cross-matching procedures whether circulating antibodies against these systems in the recipient could cause the rejection of the bone marrow graft. It appears to us to be a point of some urgency to find out whether this is so. The Seattle group and Jeannet have provided further evidence in that ^{51}Cr release studies, antibody-dependent CdC, as a sensitive indicator of cellular and humoral immunity of the recipient against the donor, correlated with graft rejection (32; Jeannet, personal communication). It is not known for which non-HLA system incompatibility was detected. Several other systems in animals have been shown to be of possible relevance for bone marrow transplantation: the Hh and Mli systems in the mouse and the colonic secretion system W/Z in the dog (7,12,34). Whether analogous systems exist in man has not been determined. In this context, it is of significance that, for the detection of all these determinants, techniques other than the CdC technique are necessary. Furthermore, the target cells in these techniques will also be different from the mononuclear cell suspension routinely used in the CdC technique. The European Bone Marrow Transplant Cooperative Group plans to make the technical facilities necessary to carry out these tests available to its members.

CAN HLA-IDENTICAL, UNRELATED DONORS BE USED IN BONE MARROW TRANSPLANTATION?

The question can be answered, in all probability, in the affirmative. But only a few of these transplants have been attempted. With the possible exception of one case, none was successful (10). But encouragement is given by a small number of mixed leukocyte culture (MLC) negative haplo-identical transplants (parent-child, siblings) from non-inbred families, several of which have been reported to be successful (6). It must be assumed that if it is possible to match successfully for one "unrelated" haplotype, it should also be possible to match for two. The lesson here is that if no HLA-identical sibling is available, one should not despair but look for other MLC-negative family members. An added advantage is that parents have a good chance of being identical for non-HLA loci.

The question then can be asked whether one should match for the SD or HLA-A, -B, and -C antigens or the MLC-stimulating determinants or both. In man, sufficient data are of course not available. Furthermore, no data are available for outbred mice and only limited data for the dog (27,31). Most critical would be studies in monkeys, which will probably soon be forthcoming.

Because bone marrow transplant data in experimental animals are lacking, it seems reasonable to ask whether other transplants in man may indicate what to expect. Table 20-4 summarizes experimental skin transplants in man, and these are a much more severe test of compatibility than are bone marrow transplants. It is clear that a negative MLC is a more effective way to improve skin graft survival than SD matching, but the combination of the two is still better. The 17-day mean graft survival that can be obtained in this manner is not much shorter than that obtained in HLA-identical siblings in our laboratory (19 days). Similar data are avail-

TABLE 20-4 Skin Graft Survival and Matching for HLA

HLA-A and HLA-B	MLC	MEAN SURVIVAL Time ± S E (days)	NUMBER
Nonidentical	+	10.0 ± 1.08	13
Identical	+	11.8 ± 0.59	20
Nonidentical	−	14.4 ± 2.07	5
Identical	−	17.3 ± 2.5	4

TABLE 20-5 The Recognition of HLA D Determinants by B Cell Serology

HLA D	ANTI B CELL SERUM	HLA D ANTI B CELL SERUM + +	+ −	− +	− −	GENE FREQUENCIES DEFINED BY HTC	Anti B cell sera
DW1	He	8	0	4	19	0 0889	0 1339
	Du	11	0	0	21		
DW2	CB	13	0	1	47	0 1287	0 1229
	RD	9	0	4	44		
DW3	Moa	27	0	0	34	0 1462	0 2033
	WH	16	2	4	39		
DW4 (+ 7)	Smith (Bodmer)	20	10	1	30	0 0669	0 1229
DW5	Pichon (Dausset)	8	5	2	45	0 0412	0 0906
DW6 (+ 2)	Po	14	4	8	35	0 0458	0 1229
LD 107	Si	15	1	0	45	0 0672	0 1451
LD 108	TL (Thorsby)	8	0	7	26	0 1039	0 0594
						0 6888	1 0010

The panel was first typed for HLA D by HTC and PLT and was then studied by sera recognizing B cell determinants The fit of the B cell typing with PLT corrected HTC typing is remarkably good for some determi nants It is clear that the sum of the gene frequencies of the HLA D determinants as established by HTC alone is much smaller than if the HLA D determinants had been studied by Bcell serology The names of the investigators who made these sera available are given in parentheses The remainder of the sera were obtained from Leiden

able for kidney grafts (5) The question is, How does one obtain unrelated donors who are identical for the HLA-A, -B, and -C antigens and MLC negative with the recipient Large numbers of donors have been HLA typed, with the intention of using them for platelet and granulocyte support therapy, and some of these donors are willing to donate bone marrow In Europe alone, the files already exceed 50,000 HLA-typed donors Thus, it would be possible to select, in many instances, for a given patient a donor who is at least HLA-A and -B matched Those donors who are MLC negative with the recipient could be further selected by direct MLC testing This is, however, a laborious procedure and when the patient has only a few lymphocytes, it will be difficult if not impossible to realize

Recently, it has become possible to type for the alleles of the strong MLC-stimulating HLA-D locus using serological techniques (22) (Table 20 5) There are only eight alleles for HLA D (30) It is quite possible that some of the HLA-D specificities will be split in two or more determinants, as was the case for the HLA-A and B locus antigens Nevertheless, as Table 20 6 shows, matching for even this small number of alleles increases MLC negativity from practically 0 percent in the HLA-D different combinations through 25 percent in the SD nonidentical, HLA-D-identical combination, to nearly 80 percent in the SD- and HLA-D-identical combinations These data confirm and extend earlier observations and prove that MLC reactivity is governed not only by loci in the HLA-D region, but also by loci near HLA-A and/or -B These findings have furthermore a number of theoretical and prac

TABLE 20-6 Matching for HLA A B and D a Outcome of the MLC Test

HLA A and -B	HLA -D	MLC STIMULAT INDEX < 2	MLC STIMULAT INDEX 2–8
≠	≠	1	65
=	≠	0	11
≠	=	15	26
=	=	10	2

tical implications From the practical point o the findings are clear cut Identity at HLA and -D implies a 80 percent chance of a n MLC The loci responsible for the remair percent could be outside HLA and should b tified From the theoretical point of view findings are less simple The one MLC n HLA-A, -B, and -D disparate combination conflicts with most accepted theories on MI ing Short of a technical error, which is ui it can only be explained by non-reactive clo was first described by Svejgaard's group (4 dividuals who are mismatched for HLA HLA-B, but matched for HLA-D, produce nificant stimulation presumably because of ences for loci identical with or near to HLA or B

It is clear that, until now, our view on the anism of strong MLC stimulation has been s tic It is even possible that an equivalent of 2 I-J locus coding for determinants on supp cells exists in man and that disparity for locus can suppress MLC activation (28) I

course, too early to know whether these findings can be used in the selection of unrelated donors for bone marrow transplantation, but it is equally clear that we ought to find out For the time being, our attitude should be one of extreme caution If at all possible, we should wait until data from Rhesus monkeys are available Until that time, only SD-identical, MLC-negative donors should be used and the CML test should also be negative

THE INFLUENCE OF SENSITIZATION ON THE EFFECTIVENESS OF IMMUNOSUPPRESSION

Gluckman et al have shown that the responsiveness of the patient, i e , his or her capacity to form immunity against major histocompatibility complex (MHC) determinants and other antigens, and the effect immunosuppression has on this immunity, correlates with bone marrow graft prognosis and especially rejection (13) The immunological capacity of both patient and donor are thus variables to reckon with, and the question whether we can influence it by other means than chemotherapy alone is thus a relevant one Although we know that anti-HLA-A, and -B antibodies in the recipient will destroy most kidney grafts from incompatible donors, it is equally certain that kidney grafts in recipients who had received no blood transfusions before transplantation have a graft prognosis that is extremely poor less than 20 to 30 percent of the grafts function at 1 year in the non-transfused group, versus 70 percent in the transfused group (17,20) Balner and his colleagues have confirmed these findings in a randomized prospective study in Rhesus monkeys (9) The unexpected observation was that whereas immunosuppression alone (standard doses of Imuran and prednisone) *after* transplantation had no effect on graft survival, the combination of immunosuppression after transplantation with blood transfusions *before* transplantation had a very significant effect (Figure 20-1)

The mechanism for this very effective graft prolongation is completely unclear (competition, suppression cell induction, enhancing antibodies?) But even if the mechanism is unknown, it might still be of interest to test whether it could be used to suppress the GvH reactivity of the donor, especially against non-HLA determinants It might be a relatively small price for the donor to pay if this could prevent GvHD in the recipient

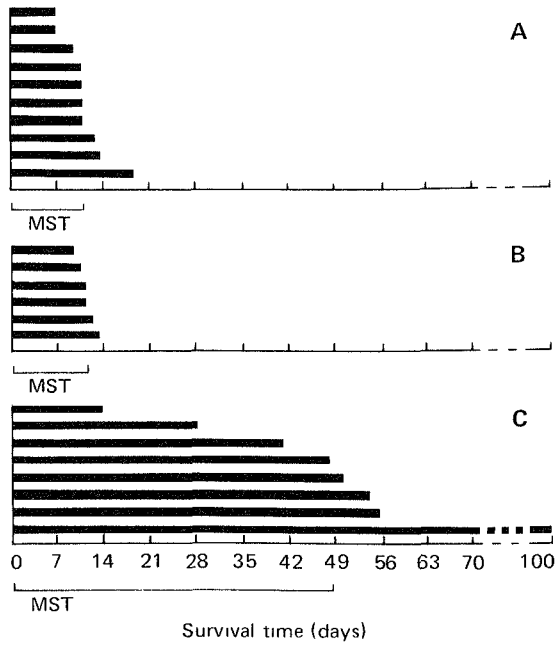

FIGURE 20-1 The influence of blood transfusion on kidney allograft survival in unrelated Rhesus monkeys (A) Non transfused/no immunosuppression mean survival time (MST) 11 4 days (B) non transfused/immunosuppression MST 11 0 days transfused/immunosuppression MST 48 8 days Reprinted with permission of *Lancet* (see reference 9)

SUMMARY

With the help of the immunofluorescence test, platelet antibodies can be detected and compatible platelets selected Such platelets, if HLA compatible, have a nearly 100 percent survival rate, even in hyperimmunized patients The use of lymphocyte-free platelets for transfusion, which can be easily obtained by three-step differential centrifugation, prevents immunization in over 70 percent of the recipients

The cell mediated lympholysis (CML) test and a complement-dependent cytotoxicity (CdC) test can be used to detect HLA-restricted, anti-H-Y immunity in some immunized women This might explain, in part, the poorer prognosis of the sex-mismatched bone marrow grafts

In the future, if HLA-identical siblings of patients are not available or suitable for bone marrow transplantation, unrelated HLA-identical donors might take their place Clinical data obtained with other organ transplants indicate that prognosis when the donor is unrelated might be comparable or better than that of HLA-identical sibling donors Thus, HLA-D-identical donors can be found relatively easily because it is now possible to type for HLA-D serologically The HLA-A, -B, and -D-identical, donor-recipient pairs are in mixed leukocyte culture (MLC) negative about 80 percent of the cases

If blood transfusions are combined with chemo-

therapy (Imuran and prednisone), very effective "immunosuppression" can be obtained in kidney transplantation It would be of interest to test whether such a procedure could be used to increase the number of successful bone marrow transplants

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