JCI The Journal of Clinical Investigation

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J Clin Invest. 1977;59(4):625-632. https://doi.org/10.1172/JCI108680.

Research Article

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Treatment of Aplastic Anemia by Marrow Transplantation from HLA Identical Siblings

PROGNOSTIC FACTORS ASSOCIATED WITH GRAFT VERSUS HOST DISEASE AND SURVIVAL

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ABSTRACT 73 consecutive patients with severe aplastic anemia were treated by marrow transplantation from hematologically normal HLA identical siblings. 68 patients lived long enough to document marrow engraftment. 21 rejected the graft and 19 of these died. 47 had sustained engraftment and 18 of these died. In 16 patients, death was associated with graft versus host disease. 29 patients with sustained engraftment are alive with complete hematologic restoration between 8 mo and 5 yr. This analysis, by using a proportional hazards regression model, was directed at identifying factors that predicted survival (and absence of graft versus host disease). Of the 24 factors entered into the analysis only two strongly correlated with survival: (*a*) sex match of donor and recipient ($P < 0.01$), and (*b*) absence of refractoriness to random donor platelets at the time of transplantation ($P < 0.05$). Refractoriness adversely influenced the survival of the sex mismatched patients. These data suggest that X and Y-associated transplantation antigen systems are important determinants of the outcome of marrow grafts between HLA identical siblings for the treatment of aplastic anemia. The mechanism by which refractoriness to random donor platelets influences survival is currently unclear.

INTRODUCTION

Marrow transplantation is an effective treatment of severe aplastic anemia provided that an HLA identical sibling can be identified as the marrow donor. Between October 1970 and November 1975, 73 consecutive patients have been treated by marrow transplantation in Seattle and 31 (43%) are alive with complete hematologic restoration between 8 mo and 5 yr (1–4). The important role of marrow grafting in the treatment of severe aplastic anemia has been emphasized by a recent prospective trial which showed it to be more effective than conventional therapy (5).

The still high mortality associated with marrow transplantation can largely be accounted for by two phenomena, both setting the stage for fatal infections: marrow graft rejection and graft versus host disease (GVHD).[1] Marrow graft rejection has recently been the subject of a separate analysis (manuscript in preparation) and was found to be associated with two prognostic factors: positive in vitro tests of sensitization of marrow recipient against marrow donor (presumably reflecting transfusion induced immunity to minor transplantation antigens), and a low number of marrow cells used for transplantation ($<3 \times 10^8$ cells/kg).

The present analysis was aimed at identifying prognostic factors associated with GVHD and survival in those patients who did not reject their marrow graft, but rather showed sustained marrow engraftment. For this purpose, proportional hazards and logistic regression models (6, 7) were applied to the data available from the patients pre and post transplantation courses.

METHODS

73 consecutive patients with severe aplastic anemia were treated by marrow transplantation from HLA identical sib-

Dr. Thomas is a recipient of Research Career Award AI 02425 from the National Institute of Allergy and Infectious Diseases.

Received for publication 23 September 1976.

[1] *Abbreviations used in this paper:* GVHD, graft versus host disease.

TABLE I

Descriptive Data on 47 Patients and Their Donors

Patient age	2–67 (median 16) yr
Sex	20 F, 27 M
Etiology of aplastic anemia	
Unknown	28
Hepatitis	3
Fanconi syndrome	3
Drug or chemical induced	11
Paroxysmal nocturnal hemoglobinuria	2
Duration of aplastic anemia	0.5–96 (median 2) mo
Preceding transfusions	44
Refractory to random platelets	18
Androgen treatment	26
Prednisone treatment	29
Relative response index*	
positive	5
negative	22
unknown	20
Donor age	4–66 (median 18) yr
Sex	31 F, 16 M
Sex matches:	22 pairs
ABO erythrocyte antigen matches	41 pairs
Conditioning for transplantation‡	
CY	31
TBI§	6
PAPAPA-CY‖	10
Marrow cell dose, $\times 10^{-8}$/kg	0.9–10.9 (median 3.3)
GVHD:¶	
Grade 0, none	19
Grade I, mild	5
Grades II–IV, moderately severe to severe	23

* Response in mixed leukocyte culture of patient cells to donor cells in relation to response to unrelated cells expressed as a ratio; the relative response index (12).

‡ CY, 50 mg cyclophosphamide/kg body weight i.v. on days −5, −4, −3, and −2; TBI, 1,000 rads midpoint tissue dose of total body irradiation on day 0; PAPAPA-CY, 12.5 mg procarbazine/kg i.v. on days −9, −7, and −5; 12 mg antithymocyte globulin/kg subcutaneously or i.m. on days −8, −6, and −4; and cyclophosphamide 50 mg/kg i.v. on days −5, −4, −3, and −2. The day of marrow infusion is designated day 0.

§ Two of these were given CY, 60, and 180 mg/kg, respectively, and TBI.

‖ One of these was given PAPAPA-TBI.

¶ For GVHD grades see references 8, 9, and 11.

lings. The definition of severe aplastic anemia, details on the selection of patients and their donors for transplantation, the conditioning regimens for transplantation with immunosuppressive agents, the grafting procedure, the clinical and histologic diagnosis of GVHD and the GVHD grading system, and each patient's pre and postgrafting course, have been described (1–4, 8–10). 5 of the 73 patients died between days −1 and 8 (days 0 is the day of marrow grafting), 1 of heart failure and 4 of gram negative bacterial infections. These five died too early for meaningful evaluation and were not considered in the present analysis. In addition, 21 patients rejected their initial graft and only 2 of them survived. In contrast, there were only 18 deaths among 47 patients who had sustained engraftment. GVHD was not a problem in patients with graft rejection while it frequently occurred in patients with sustained engraftment. It seemed reasonable, therefore, to deal with these groups of patients separately in attempts at identifying prognostic factors.

Table I summarizes some of the data used on the 47 evaluated patients. All patients were given intermittent treatment with the immunosuppressive agent methotrexate within the first 100 days of grafting to prevent or ameliorate GVHD. In addition, 15 patients received rabbit, goat, or horse antithymocyte globulin in an attempt at treating established GVHD. All had sustained hemopoietic engraftment as documented by daily monitoring of peripheral blood counts and frequent determinations of marrow cellularity, monitoring of blood genetic markers, and by cytogenetic analysis when donor and recipient were of opposite sex.

RESULTS

Survival and causes of death. 29 of the 47 patients are surviving between 8 and 62 mo after transplantation with complete hematologic restoration. 5 of these have chronic GVHD either in skin or liver with reduced activity in 3, and 24 patients have no problems. 18 patients have died: 12 died with grade II-IV GVHD, 8 of bacterial or fungal infection, and 4 of interstitial pneumonia. Five additional patients died with interstitial pneumonia and four had grade II-IV GVHD which had resolved under therapy with antithymocyte globulin. Thus, in 16 of the 18 patients moderately severe to severe GVHD (grade II-IV) was present before or at death. One additional patient died of unknown causes on day 427 without preceding GVHD.

Analysis of factors associated with survival. Table II shows the number of deaths and the total number of patients studied in relation to some of the factors used in the analysis. In view of the variable follow-up duration among patients, the information on the number of deaths was supplemented by information on the exponential estimated means of survival time. This is the ratio of the total cumulative survival time over the number of deaths in a given group of patients. Because of a small number of deaths in some groups of patients, the mean survival estimate may exceed the time any patient has been followed. This suggests caution in interpreting the mean survival time estimate. Here is was used only for descriptive purposes in preparation for the more comprehensive regression analysis that follows.

Table II shows that a number of factors had a possible association with survival duration after marrow grafting. Other factors included in the analysis

TABLE II

Incidence of Death among 47 Patients (Pts) with Sustained Marrow Grafts, Classified by Various Factors

Aplastic anemia

	Duration, *mo*				Etiology				
	<5	5–10	10–30	>30	Hepatitis	Fanconi	Chemical	PNH*	Unknown
No. pts dead/ no. pts studied	11/31	4/9	2/4	1/3	1/3	2/3	6/11	0/2	9/28
Mean survival‡	48	46	36	50	64	20	21	78	58

Preceding treatment

	Total units of blood or platelets§					Androgens		Prednisone	
	0	1–20	21–40	41–90	>90 (or multiple)	Yes	No	Yes	No
No. pts dead/ no. pts studied	0/3	4/12	6/10	4/12	4/10	13/26	5/21	12/29	6/18
Mean survival	89	59	15	61	44	33	81	40	59

Donor-recipient matching characteristics

	Sex match		ABO match		Age difference/ donor age minus patient age, *yr*			RRI‖		
	No	Yes	No	Yes	>−2	(−2,3)	>3	<1.6%	>1.6%	Unknown
No. pts dead/ no. pts studied	16/25	2/22	3/6	15/41	6/13	5/11	7/23	7/22	2/5	8/20
Mean survival	20	257	23	51	54	27	58	41	35	65

Marrow transplantation characteristics

	Conditioning regimen¶			Marrow dose, × 10^{-8} cells/kg				
	CY	TBI	PAPAPA-CY	0–1.9	2.0–2.9	3.0–3.9	4.0–5.9	6.0
No. pts dead/ no. pts studied	8/31	5/6	5/10	4/6	8/13	2/7	2/11	2/10
Mean survival	80	11	27	21	27	54	114	99

Other patient data

	Sex		Age, *yr*					Refractory** to random platelets	
	Female	Male	0–9	10–19	20–29	30–39	>40	No	Yes
No. pts dead/ no. pts studied	6/20	12/27	2/12	5/18	2/4	3/7	6/6	9/29	9/18
Mean survival	63	38	105	81	40	37	4	63	29

* PNH, paroxysmal nocturnal hemoglobinuria.

‡ Months (total accumulated survival time/number of deaths; in the case of zero deaths number given is total accumulated survival time).

§ From family or random donors.

‖ RRI, relative response index in mixed leukocyte culture (12).

¶ See Table I for details.

** Refractoriness to random donor platelets was defined on clinical grounds and assumed to be present whenever platelet transfusions failed to raise the platelet counts above expected levels (in the absence of consumption).

TABLE III

Proportional Hazards Regression Analysis of Survival Times of 47 Patients with Sustained Marrow Engraftment

	Coefficient (b)	Standard error	Ratio	
Sex match, 0 matched, 1 not matched	2.420	0.862	2.81 ($P < 0.01$)	
Refractory to random platelets, 0 no, 1 yes	1.298	0.599	2.17 ($P < 0.05$)	
Marrow dose, 10^{-8} cells/kg	−0.005	0.022	−0.22	NS
Androgens, 0 no, 1 yes	0.629	0.638	0.99	NS
Patient age, *yr*	0.282	0.198	1.42	NS
ABO match, 0 no, 1 yes	0.700	0.828	0.85	NS
Conditioning regimen, 0 CY,* 1 other	0.578	0.546	1.06	NS
Patient sex, 0 female, 1 male	0.102	0.602	0.17	NS

* CY, cyclophosphamide.

but excluded from Table II for brevity were the direction of the sex match (F → F, M → M) or mismatch (F → M, M → F), donor age and sex, donor and recipient ABO groups, transplantation year (1970/71, 1972, 1973, 1974, and 1975), and numbers of individual units of random donor whole blood and of random or family donor platelets transfused. A good prognosis appeared to be associated with each of the following eight factors: the absence of androgen treatment, patient of female sex, donor-patient sex match, ABO erythrocyte group match, cyclophosphamide as conditioning regimen, larger number of marrow cells infused, young patient age, and lack of refractoriness to platelet transfusions from random donors. Some of these associations were likely to be spurious, and it was necessary to use a multifactor approach to the analysis. The response was the duration of survival along with whether or not the patient was alive at the closing date for this analysis (28 February, 1976). A number of approaches to such analyses have been developed over the past decade. One of the most flexible is that based on the proportional hazards model (6). This approach presumes the death rate (hazard rate, force of mortality, and instantaneous failure rate) to be a multiplicative function of prognostic factors and to be otherwise arbitrary. More specifically, the logarithm of the death rate can be written

$$zb + g(t)$$

where z is a (row) vector of prognostic factors, b a (column) vector of coefficients, and g an arbitrary function of failure time t. This model was applied to date on the 47 patients with the eight factors listed in the preceding paragraph, utilizing the estimation procedure described in (6). The results (Table III) indicated that sex match ($P < 0.01$) and nonrefractoriness to random platelets ($P < 0.05$) both predicted good survival. Both M → M and F → F transplants had a good prognosis while both F → M and M → F transplants had a bad prognosis. The other factors failed to correlate significantly with survival after taking account of these two factors. Further, transplant year and duration of aplastic anemia before transplantation did not correlate significantly when added to the regression equation. It should be remembered, however, that the regression analysis involved rather small sample sizes (47 patients, 18 deaths) and that factors such as preceding androgen treatment and age should be monitored further. At the moment, however, the association of such factors suggested by Table II is largely accounted for through a correlation of these factors with sex match. For instance it happened that 67% of the patients under 10 yr of age were sex matched as compared to only 31% of those 30 yr of age or older. Table IV and Fig. 1 show the very dominating effect of sex match on survival with death of only 2 of 22 sex matched patients. Refractoriness to random donor platelets was important for those patients who were sex mismatched with their marrow donors (Table IV).

Analysis of factors associated with GVHD. Table I and the more detailed summaries presented elsewhere (1–4) show that GVHD was a major problem in patients with sustained engraftment. 28 (60%) of the 47 patients under consideration had clinically detectable GVHD and 19 did not. In 5 of these GVHD was mild (grade I) and in 23 it was moderately severe to severe (grade II-IV).

TABLE IV

Association between Survival, Sex Match, and Refractoriness to Random Donor Platelets

	Refractory to random platelets	
	No	Yes
	No. of patients alive/ no. of patients studied	
Sex match		
F → F	8/8	4/5
M → M	4/5	4/4
Total	12/13	8/9
Sex mismatch		
F → M	6/12	1/6
M → F	2/4	0/3
Total	8/16	1/9

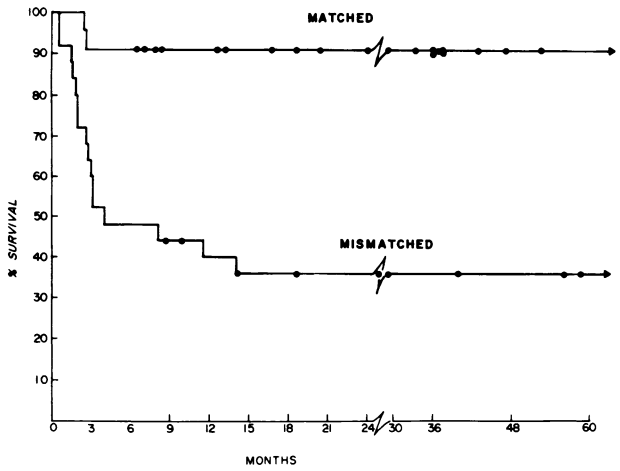

FIGURE 1 Survival of 47 patients with severe aplastic anemia who had successful and sustained marrow grafts from HLA identical siblings. The survival of 22 patients with sex matched donors is compared to that of 25 patients whose donors with sex mismatched. Dots indicate surviving patients (as of 1 May, 1976).

Grade I GVHD is transient and the diagnosis cannot always be made definitively. For the current analysis, therefore, we combined GVHD grades O and I and compared them to grades II–IV by using the same factors as in the survival analysis above. Again, some suggested associations were found (Table V). An increased incidence of GVHD (grades II–IV) was seen in patients who were sex or ABO mismatched with their donors and in patients who had been previously treated with androgens or were refractory to random donor platelets. Also, male patients seemed to have a higher incidence of GVHD.

When these factors were entered into a binary logistic regression analysis (7) only sex mismatch showed a significant association with presence of grade II–IV GVHD ($P < 0.01$) though the exclusion of some other factors may simply reflect small numbers. Table VI shows the association of both sex match and GVHD grade to patient survival. The favorable influence of sex match on survival cannot be entirely explained in terms of absence of GVHD. Similarly, sex mismatch appears to have an unfavorable influence on survival that cannot be entirely explained by increased incidence of GVHD alone.

DISCUSSION

The results of the current study indicate that the occurrence of GVHD and survival of patients with aplastic anemia treated by marrow transplantation are strongly influenced by whether or not marrow donor and recipient are matched for sex. This strong association between sex match and survival (and absence of GVHD) remained highly significant ($P < 0.01$) even after consideration of all other factors entered into the proportional hazards regression analysis. Good survival was associated with an F → F and M → M match while poor survival was seen both with an F → M and M → F mismatch. These findings implicate histocompatibility genes on the human X and Y chromosomes as important determinants for the outcome of marrow grafts between HLA identical siblings.

Calculations of the chances of incompatibility for a hypothetical X-associated biallelic system suggest the following frequencies of incompatibilities (expressed as percent of the heterozygous frequency): M → F = 125%, M → M = 50%, F → M = 25% and F → F = 25%. Additional consideration of a Y-associated antigen system increases incompatibility in the F → M combination to 100%.

Both X and Y associated transplantation antigens (H-Y, N-X) have been described in mice. The first observation on H-Y was made by Eichwald and Silmser in 1955 (13). They found that intrastrain A/Jax mice skin grafts regularly failed when the donor was male and the recipient female. In contrast, M → M, F → F and F → M grafts always succeeded. These ob-

TABLE V
*Incidence of GVHD (Grades II–IV) among 47 Patients with Sustained Engraftment**

Donor-recipient matching characteristics						
Sex match			ABO match			
No	Yes		No	Yes		
17/25	6/22		4/6	19/41		
Other patient data						
Sex			Androgens		Refractory to random platelets	
Female	Male		No	Yes	No	Yes
8/20	15/27		8/21	15/26	13/29	10/18

* Number of patients with grade II–IV GVHD/number of patients studied.

TABLE VI
Association between Survival, Sex Match, and GVHD Grade in 47 Patients with Sustained Engraftment

Sex match	Total	GVHD grade				
		0	I	II	III	IV
		No. of patients alive/no. of patients studied				
Yes	20/22	13/13	3/3	2/2	1/1	1/3
No	9/25	4/6	1/2	2/4	2/9	0/4

servations on skin engraftment were subsequently confirmed and extended by others (14–20). In addition, Lengerova and Chutna in 1959 carried out marrow grafts after 900 R total body irradiation in C57B1 mice and found that survival of males given female grafts was inferior to that of all other combinations (21). The causes of death of the mice in these studies, however, were not clearly described. One other group of investigators was unable to confirm these findings (22). Parabiotic union of specifically sensitized C57 females with males of the same strain, however, resulted in parabiotic intoxication of 50% of the latter (14). Uphoff treated (C3Hf × NBL) F_1 recipients with marrow grafts from both male and female reciprocal hybrid donors and reported 50% mortality from GVHD at 2 mo (23). She attributed GVHD to H-Y antigens.

It was noted that the female response to H-Y varied depending on the strain used (15, 17, 18, 20, 24–26). An early response to H-Y was associated with H-2 haplotypes b and s, an intermediate response with haplotypes k, d, i, and h, and late or absent responses with H-2 haplotypes a and f. The H-2 linked gene determining male skin graft rejection is presumed to be either distally linked to or within the region containing K and Ir-l. Although the H-2 linked gene has a dominating influence on the response to H-Y, minor genetic factors or background effects probably contribute as well. This assumption is based on the finding of two different grades of immune responses to H-Y among six H-2b bearing mouse strains (20, 24–26).

The first observation of histoincompatibility associated with the X chromosome in mice was made by Bailey in 1963 working with two highly inbred strains of mice (C57B1/6 JNBy and BALB/c AnNBy) and their reciprocal F_1 hybrids (27). He not only observed that incompatibility for the X chromosome lead to skin graft rejection, but also found differences in survival of grafts from the reciprocal hybrids indicating that the antigenicity determined by the gene on the X chromosome of the one strain was stronger than that of the other strain. Bailey's findings were subsequently confirmed by other investigators (28–30). It is interesting that Lengerova and Chutna (21) observed death of some C57B1 females given intrastrain male marrow grafts.

Overall, the X and Y associated transplantation antigens in mice appear to be weak as indicated by slow skin graft rejections. Similar weak male specific transplantation antigens have been reported in rats, rabbits, and platyfish (31–33). In contrast, our observations suggest that X and Y associated transplantation antigens in man are rather strong and cause GVHD and increased mortality.

A second important prognostic factor in the present study was refractoriness to random donor platelets at the time of transplantation. 40% of our patients were refractory (2, 4). Refractoriness was independent of the duration of aplastic anemia and the number of transfusions before transplantation and showed a significant association with mortality after transplantation ($P < 0.05$). Refractoriness adversely influenced only the survival of those patients who were sex mixmatched with their donors. It has to be remembered that refractoriness need not always reflect immunological refractoriness, the product of transfusion-induced sensitization to random platelets, but also can be the result of platelet consumption, e.g. in infection. Since 52% of our patients were infected at admission (2, 4), the distinction between immunological and consumptive refractoriness was not always easy to make. Still, our observations are intriguing. We at first assumed that the influence of refractoriness on survival was due to differences in postgrafting transfusion support among the patients, i.e. differences in exposure to major and minor histocompatibility antigens on leukocytes and platelets in the transfused blood triggering varying degrees of immune responses of donor cells against host antigens. Statistical analysis of the data, however, (separately considering transfusions from the marrow donor, other family members and random donors) failed to show significant differences between the various groups of patients (refractory and nonrefractory, sex matched and mismatched).

Another speculative explanation for the influence of refractoriness on survival involves the immune responsiveness of the marrow donor. As discussed earlier, immune responsiveness to the H-Y antigen in mice seems to be mainly associated with certain H-2 alleles although genetic background effects have also been observed. It has been speculated that early or late rejection of male skin by the female mouse may reflect general efficacy of the immune system rather than reactivity to a specific class of antigens. Refractoriness to random donor platelets could be the expression in man of strong general immune responsiveness as determined by Ir genes within the major histocompatibility complex. Since marrow donor and recipient were identical for the major histocompatibility complex, strong immune responsiveness seen in the recipient might also be present in the donor. In the case of a sex mismatch, strong immune responsiveness would be directed against X and Y-associated transplantation antigens resulting in a high incidence of GVHD and death. If this speculation were true and linkage disequilibrium between various loci of the major histocompatibility complex, is assumed, an association between refractoriness and certain HLA antigens might be expected. However, no significant deviation of HLA allele frequencies was

seen among the small number of refractory patients nor among the patients with acute or chronic GVHD.

Our results differ from the results of an analysis carried out by the bone marrow transplant registry (34). They reported that the duration of aplastic anemia before transplantation, the number of pretransplant transfusions, and the age of the recipient correlated with survival. At least four factors may account for these differences: (*a*) the registry analyzed data from 17 transplant teams, data which are probably not derived from a consecutive series of patients and could be the result of selective reporting (patients from Seattle were not entered into the registry); (*b*) the registry did not differentiate between patients who rejected and patients with sustained engraftment. As discussed earlier, graft rejection in our series of patients was clearly associated with two different prognostic factors, a positive relative response index and low marrow cell dose. Mortality among patients with graft rejection was very high and never associated with GVHD while mortality among patients with sustained engraftment was lower and frequently associated with GVHD. Hence, it seems reasonable to analyze the two groups of patients separately; (*c*) the registry did not carry out multifactorial analyses which are more likely to isolate those factors that are really important for the success or failure of marrow transplantation; and (*d*) results of in vitro tests of sensitization, such as the relative response index, were not entered into the registry analysis.

In view of the still small number of patients in our study, our results should be interpreted with caution. If true, they have some obvious therapeutic implications, e.g. selection of a sex matched marrow donor if several HLA identical siblings are available, and early transplantation (*a*) becuase of the excellent chances for survival in the case of a sex matched recipient, and (*b*) to prevent further deterioration of the chances for survival in the case of a sex mismatched recipient by avoiding the development of refractoriness. Knowledge of the association of antigens important for the development of GVHD with the X and Y chromosomes in man might lead to the development of in vitro typing techniques to detect these antigens. Availability of such typing techniques will facilitate the selection of donor recipient pairs for marrow transplantation in aplastic anemia.

ACKNOWLEDGMENTS

This investigation was supported by grant nos. CA 18029, CA 15704, CA 05231, CA 18221, CA 17117, and CA 18579, awarded by the National Cancer Institute, Department of Health, Education, and Welfare, and contract AI 52515 from the National Institute of Allergy and Infectious Diseases.

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