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CONTRIBUTION OF BONE MARROW TRANSPLANTATION TO KNOWLEDGE OF HISTOCOMPATIBILITY AND ROLE OF PRESENSITIZATION

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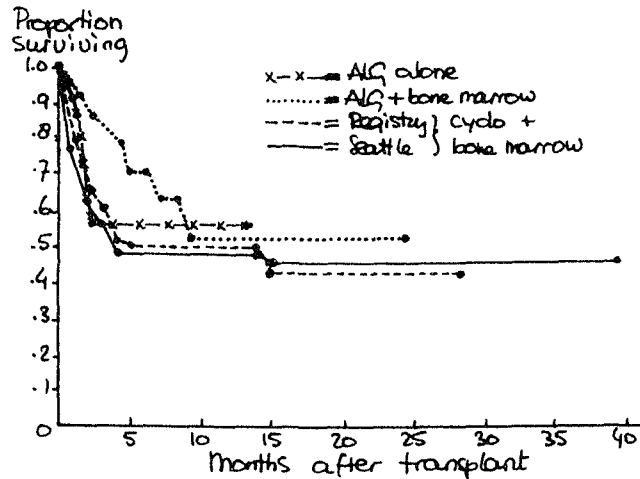
Allogeneic bone marrow transplantation has become an accepted form of treatment both for aplastic anaemia and acute leukaemia and of course for immune deficiencies where it was first successfully applied. However, the main indication remains aplastic anaemia and leukaemia and the results over the last few years have not changed much.

About half of the patients will recover, the treatment will be for them successful and about half of them will die. This is not only unfortunate for the patients but perhaps even more frustrating is that we still do not know which patients will die and which will survive. We do know that the graft versus host disease and interstitial pneumonia are the main cause of death but we are as yet unable to identify the factors which initiate these complications. The fact that monozygotic twins have far less of these complications indicate that immunogenetics play a role. Identifying that role is further complicated by the fact that non-immunogenetic factors such as age, the sex difference between donor and recipient, the rate of irradiation play a role as well.

Three topics will be discussed here: 1. presensitization and supportive care; 2. incompatibility for non-MHC factors detected by antibodies; 3. incompatibility for non-MHC factors detected by cellular typing.

The first topic concerns the problem of supportive care. Supportive care is extremely important for several reasons but especially so because if supportive care is optimal, it is not always necessary to perform a bone marrow transplantation.

Figure 1 shows four groups of patients suffering from aplastic anaemia, a part of which has been given a bone marrow transplant after pretreatment with cyclophosphamide. The other patients, also suffering from aplastic anaemia, have been treated by ALG alone or ALG and bone marrow and their graft survival is as good as that of the transplanted patients. There are several studies both in Europe and in the States which support this and it can be said that half of the patients suffering from aplastic anaemia will improve significantly and become independent of transfusion if given ALG but this is only possible if one has optimal supportive care.



And here we are confronted with the problem of immunization. Of course prevention of immunization is best and this is possible if one is careful not to transfuse any lymphocytes. Red cells can be given after filtering them through cotton wool filters which will retain all lymphocytes and such red cells will not immunize the patient. The remaining problems are then the platelet transfusions which often contain a substantial number of lymphocytes. Simply through one extra second centrifugation one can remove these lymphocytes and in that way sensitization of the patient becomes far less of a problem.

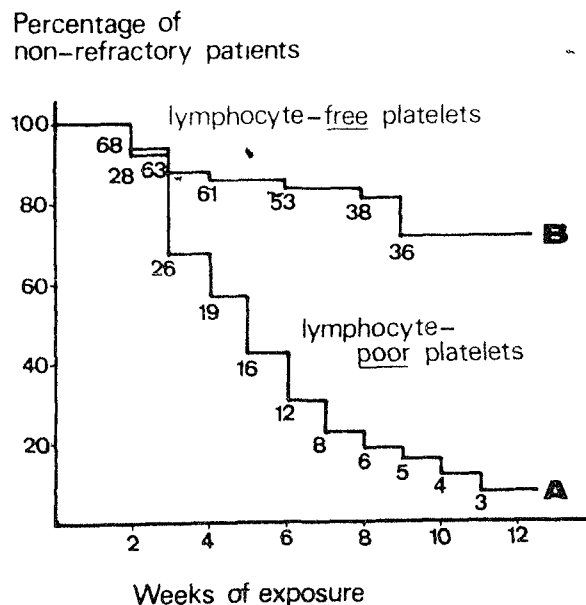
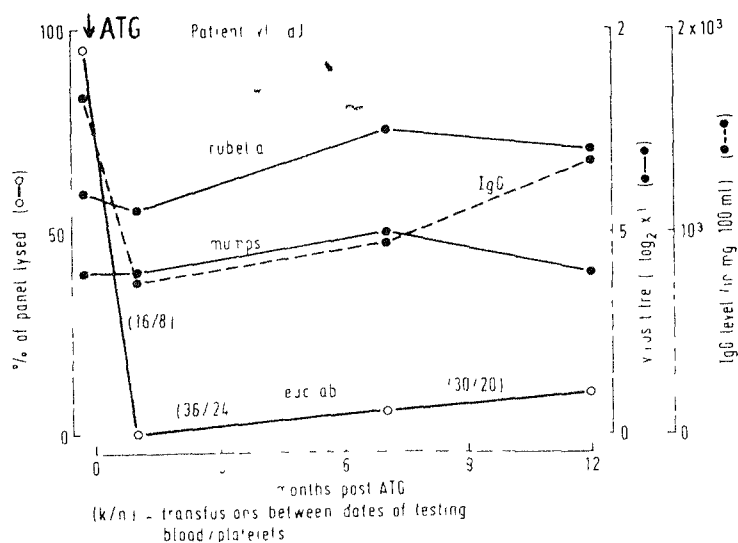


Figure 1. Comparison of the incidence of refractoriness after platelet transfusion in 28 patients who received standard platelet transfusions (Group A) and 68 patients who received leukocyte-free platelet transfusions (Group B). Numbers under the lines indicate patients at risk. (Eernisse & Brand 1981)

The results of a study performed by Eernisse and Brand are shown in figure 2. (1) Patients who received platelet transfusions which are lymphocyte-free through one single extra centrifugation remain for the larger part non-refractory, not only after three months but after a much longer period of time as well.

So the first lesson is that in supportive care, prevention is best and that one should try to keep the patient clean, that is that no HLA-antibody formation should be induced and this is possible by giving red cells and platelets which are lymphocyte-free. However, what to do if the patient becomes immunized. Of course one can give compatible platelets, but there is another possibility, which, although still experimental, is of sufficient interest to be mentioned here. Sabbe et al. (2) described that in patients with leukocyte antibodies who received ATG for the treatment of aplastic anaemia, the leukocyte antibodies disappeared while other antibodies remained unchanged. Figure 3 shows a patient who received ATG on day 0. At that time he had strong leukocyte antibodies which reacted with almost 100% of the panel. After about one month time, the leukocyte antibodies had disappeared and remained very low even although he received many blood and platelet transfusions. These however were free from lymphocytes. It is remarkable that there is hardly any fall in titer of antibodies against Rubella and Mumps and the total immunoglobulin level remains more or less normal.



[Figure 3. Evolution of total IgG content and specific antibodies to allogeneic lymphocytes and two endemic viruses after therapy with ATG. (Sabbe et al. 1981)]

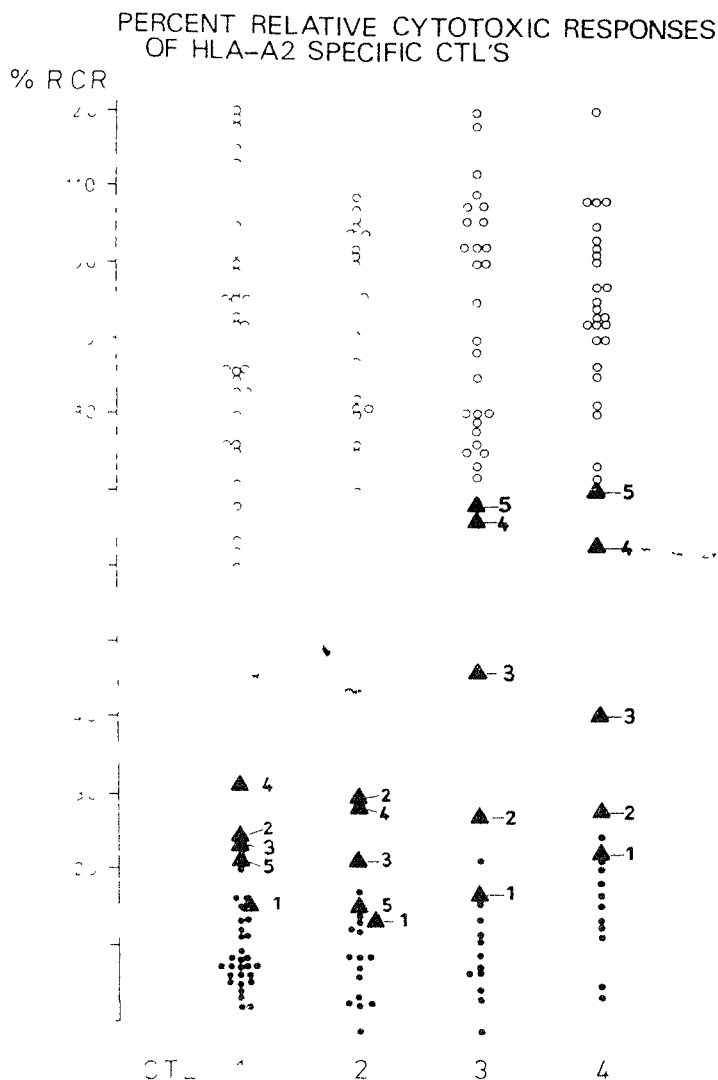
We have observed this in three other patients and this led to a randomized prospective study which was performed by Claas et al. from our group. Mice were first immunized so that they had formed leukocyte antibodies and then they were given ATG. In the animals that received ATG the antibodies had disappeared after 45 days and in the control a significant amount of antibodies had remained. The mechanism of ATG suppression of antibody formation is as yet unclear. It might well be that anti-thymocyte globulin contains a large number of anti-idiotypic antibodies and that these anti-idiotypic antibodies are able to either kill the plasma cell while it secretes the leukocyte antibodies, or stop plasma cells in another way from making such antibodies.

Let us next have a look at the non-MHC factors as they can be detected by antibodies. These are especially important with aplastic anaemia patients because these receive so many transfusions. Antibodies will be formed not only against HLA but also against granulocytes, specific, platelets, specific and many other antigens. HLA antibodies as a rule can be considered to be no problem if an HLA identical sibling bone marrow donor is available and can be used. But the situation is different for the non-MHC factors. In the earlier days of bone marrow transplantation, and especially in the treatment of aplastic anaemia, rejection was still a problem. This has nowadays been overcome by more effective pretransplant conditioning regimes and it is rarely more seen. However, in the earlier days rejection was a problem, and in a combined study with the group of Seattle, we found a significant correlation between the presence of monocyte antibodies and rejection (3). Recently these data have been confirmed by Gluckmann et al. (4). They performed a systematic study to see whether there was a correlation between the occurrence of GVH, and monocyte antibodies, but such a correlation could not be found. Although monocyte antibodies against non-MHC loci might have played a role in the past by inducing bone marrow rejection, today they do not seem to be very important.

Still, we should not forget the possibility that this all might be due to the lack of adequate information. Our knowledge of the immunogenetics of the monocyte antigens is really very limited. We do know that several loci are involved which code for these determinants and data from Moraes and Stastny (5) indicate that some of these loci might go along with the MHC and that other loci lie outside the MHC region. As the number of the loci and the number of alleles are unknown typing for these antigens is not yet possible and thus matching is not possible as well. Unless we will be able to do so, we really do not know

whether incompatibilities for these monocyte systems might play a role in GVH.

The last topic concerns the question whether typing donor-recipient pairs with cytotoxic lymphocytes could be helpful in preventing graft versus host disease. Now CTL typing can be performed in two ways, one of these is by using allo-CTL's. Such allo CTL's have been extremely useful in picking up biochemical variants of ~~HLA~~ HLA antigens which so far have not been detected by serology. Unrelated individuals are selected which differ for only one antigen, for instance A2 and in the standard CML assay A2 specific CTL's can be induced. Van der Poel (6) from our group typed about 60 people with 4 different A2 specific CTL's and figure 4 shows that one can easily differentiate between the positive results, above 60% relative killing and those which were negative, generally under 30%.



(HORAI et al 1982)

Figure 4. Percent relative cytotoxic responses of HLA-A2 specific CTL's. Open circles represent HLA-A2 positive target cells, closed circles represent HLA-A2 negative target cells. The black triangles indicate people which typed serologically A2 positive but fail to react or reacted very weakly with the CTL's. The outlier HLA-A2 positive target cells LV1-LV5 are numbered 1,2,3,4 and 5 respectively.

Further studies in which these cells were used as stimulators and responders have indicated that there are at least two variants and very recent biochemical data collected together with Ploegh (manuscript in preparation) has confirmed that they are variants very much like the M7 variant described by Biddison (7). These allo CTL's, however, will not be very useful in bone marrow transplantation for the simple reason that although they are indeed able to recognize variants in class I antigens, such variants segregate with HLA haplotypes and for that reason HLA identical siblings will always be identical for the variants. ~~The situation is~~ different with what we will call the auto CTL's which are really nothing more than the well known Doherty-Zinkernagel phenomenon (8).

In patients suffering from aplastic anaemia, Goulmy et al. (9) found cytotoxic lymphocytes which were MHC restricted and directed against H-Y. The first case, Mrs. Re, concerned a woman who suffered from aplastic anaemia and who received a large number of blood and platelet transfusions and then it was found that her lymphocytes were able to kill the lymphocytes of her brother. A further study showed that the cells of this woman killed all A2 positive male cells and virtually none of the female A2 positive cells; A2 negative cells were not killed at all.

This was a typical example of an anti-H-Y/A2 restricted cytotoxic lymphocyte, and Goulmy (10) has found since then several other examples and other workers found them as well (11,12). Here we will refer to such MHC restricted CTL cells as auto CTL's because Mrs. Re her cell was A2 positive but she killed A2 positive targets on the condition that they carried also the non-MHC determinant H-Y. What we call here auto CTL's, react with class I antigens and H-Y and for that reason do not always segregate with HLA. Of course if you study a family in which all the children are males, CTL will segregate with the HLA haplotypes.

Although these findings have been confirmed, it is uncertain to which extent they are really of clinical importance. It is a fact however that female donors lead more often to complications than male donors. However in such patients a clear correlation with the presence of MHC restricted HY CTL's has not been found although it should not be forgotten that perhaps they have not been looked for hard enough, or with the wrong kind of techniques. The clinical importance of these auto-A2 restricted anti-HY CTL's remains thus open.

Although the clinical importance of the auto or MHC restricted CTL's which are directed against the HY deter-

minants is still, apart from statistical grounds, unclear, this might be different for a newly detected non-MHC determinant. Recently a patient (designated HA) was transplanted because of acute myeloid leukaemia and received a bone marrow transplant from an HLA identical sister. Their HLA type was A2, B27, Bw62, Cw1, Cw3, DR1, DR4, MLC and CML were negative and there was a good take. Then a severe late chronic graft versus host disease set in. Goulmy et al. (13) studied this patient by using his cells after transplantation as responder cells. Table 1 shows that when posttransplant effector cells of the patients are taken, the cells of the patient pretransplantation were strongly lysed. After transplantation they were not and the bone marrow donor was also not lysed, so the conclusion is that the bone marrow donor saw something on the patient's cells which **was absent** from her cells. The cells recognized a polymorphism in unrelated individuals.

TABLE 1. Percentage lysis obtained with posttransplant effector cells of patient HA.

target cells	% lysis
patient HA (pretransplant)	+ 59
patient HA (posttransplant)	- 1
bone marrow donor	- 3
unrelated individual A	+ 5
unrelated individual B	- 7
unrelated individual C	+ 26
unrelated individual D	+ 35

(Goulmy et al. 1982)

Next the family of the patient was studied (figure 5). Both parents were killed by the CTL's of the patient taken posttransplantation. The patient before transplantation was positive, the donor negative, and of the three haplo-identical, two were killed and one was not. So in this one family we have two examples in which HLA identical siblings were differentiated using CTL typing. To test the specificity of the CTL a large panel of over 100 people has been typed and table 2 shows a part of the results. There was a clear correlation with A2. All A2 positive cells were killed on the condition that they were not a variant, the 5 negatives were the variants as discussed earlier. The only two cells which were to the best of our knowledge no variants and were not killed, were the bone marrow donor and the haploidentical sister. But the lymphocytes of the patient contained also CTL's which segregated with a split of B27 and Bw62.

We think that we are here confronted with a situation which is very similar to that of HY with a number of important differences. In the first place this minor HA antigen, the recognition of which is restricted by either HLA-A2,B27, or Bw62 might have a very high frequency, at least as far

A2 positive people are concerned.

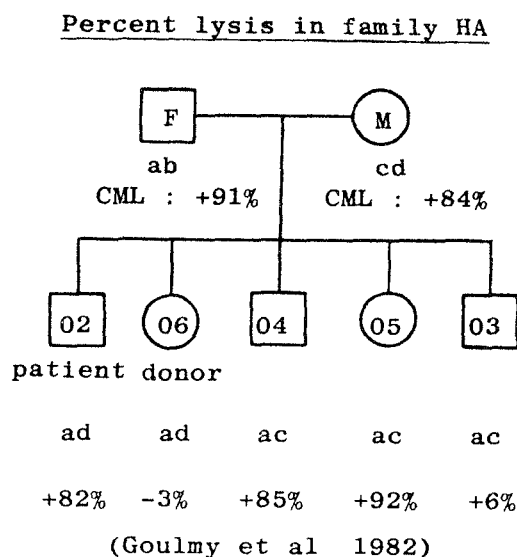


Figure 5.

TABLE 2. Analysis of HLA restricted anti-minor HA antigen lysis.

HLA serotyping of target cells					
	HLA-A2	B27	Bw62	Others	
CML	+	38	2	7	0
	-	5	5	3	44

(Goulmy et al. 1982)

Whether this minor antigen is localised on the 6th chromosome we do not know. If it is on chromosome 6, it is localised on the A side as has been shown by crossover families. It is yet unknown whether the clones which are restricted to B27 and Bw62 are directed against the same HA antigen as the A2 restricted clone, but it seems to be the most likely explanation. Using such cells we will type retrospectively all our bone marrow donors and recipients which are available and which are either A2, B27 and Bw62 positive to see whether there is a correlation with graft versus host disease and discrepancies, incompatibilities, for this minor HA antigen.

These studies show that both auto CTL's and allo CTL's react with part of an HLA class I antigen. Allo CTL's do but auto CTL's do not always segregate with HLA and for

[That reason the auto CTL's are more interesting to use in the study for bone marrow transplantation. Typing with auto CTL's might provide insight in the pathogenesis of GVH disease and in any case they will give us further insight in the immunogenetics.

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Fig. 1. Percent relative cytotoxic responses of HLA-A2 specific CTLs.

Open circles represent HLA-A2 positive target cells. Closed circles represent HLA-A2 negative target cells. The outlier HLA-A2 positive target cells LV1-LV5 are numbered 1, 2, 3, 4 and 5 respectively.

HLA phenotypes of LV1 to LV5:

LV1:	A1	A2	B8	Bw50	-	Bw6	Cw6	Cw7	DR3	DR6
LV2:	A2	Aw29	B7	Bw58	Bw4	Bw6	-	-	DRw6	-
LV3:	A2	A26	B27	B37	Bw4	-	Cw2	Cw6	-	-
LV4:	A2	A3	B8	Bw55	-	Bw6	Cw4	-	-	-
LV5:	A2	Aw32	Bw44	Bw50	Bw4	Bw6	Cw6	-	DR1	DR7