Tokai J Exp Clin Med., Vol. 10, No. 2, 3, pp. 207–214, 1985

Use of Donors Sharing One Genetic Haplotype for Bone Marrow Transplantation

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Matched sibling transplants enjoy over 95% survival of the grafting procedure, but are only available for 1:5 patients. A sibling sharing one genetic haplotype is today our next choice of donor (67% survival) faring better than other relatives (50% survival), providing total body irradiation (of the thymus) has been avoided. The latter, without increasing the attack rate (64%) of GvHD more than doubles the deaths (57% as against 27%) attributable to it.

Rejection is avoided by (a) suicide of host responders to donor buffy coat; (b) Cyclosporin-A; (c) displacement induction; (d) a higher dose of marrow.

Prevention of GvHD is essential, using either Cyclosporin-A or removing donor T-cells from marrow prior to infusion or, probably better, both. Autoblast immunisation should be further explored.

Tolerisation seems an active process, easier in the very young, and nonirradiation of the thymus is believed important. An assay to assess tolerisation (to guide cessation of immunosuppressive measures) is badly needed.

Selection of a donor whose lymphocytes can deal with intracellular infections of the host's fibroblasts is now possible.

The required increased immunosuppressive measures appear to increase the risk of leukemic relapse, and perhaps should be first improved in the more cost-effective fields of inborn error transplants.

INTRODUCTION

In Britain, one in five of the patients we would consider treating by bone marrow transplantation does not have a matched sibling donor. Among the alternatives, the use of donors sharing one-genetic haplotype (HS) with the patient has become increasingly feasible with advances in immunosuppressive therapy (Cyclosporin-A, especially) and measures designed to remove previously competent T-lymphocytes from the donor marrow prior to infusion, in the hope that the new young T-cells will have a greater chance to become tolerised to their new host, and certainly, using Cyclosporin-A it is clear that if the thymus is still present and functional, it assists this latter process. For that reason, we will report in this paper the important differences between irradiated and non-irradiated patients.

It was found in 1970 (19) and in 1972 (9) that leukocytes sharing a genetic haplotype could transfer immune function to a patient's cells, better than could leukocytes from an unrelated donor. This method was used consciously by us to switch on competent immune reactions for deficient patients, and unconsciously probably has contributed to some of the Seattle success where it has been standard practice to administer donor's leukocytes during bone marrow transplantation (BMT). Such cooperation between immunocompetent cells and the tissues of the host has been elegantly demonstrated by Zinkernagel (20).

The objectives, and problems, of using HS donors can therefore be summarised in Table 1.

Probably the first intended success following the use of an HS donor in BMT was undertaken in September 1972 (8) and the anticipated graft-versus-host disease (GvHD) was aborted

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by the elective use of Methotrexate and antilymphocyte globulin (ALG) on Days 5–7 post-graft; this was probably the first intended use of ALG in BMT; the patient survived a severe GvHD with liver involvement, the Bilirubin reaching 42 mg/100mℓ, but showed a complete recovery with development of fully normal immune function which has kept him perfectly fit to the present day; he has acquired the Rhesus type of his father as evidence of engraftment. It was almost as if the competent T-cells of the donor were removed in vivo Days 5–7, and in the patient who had been a lymphopaenic SCID with no thymic shadow visible on Chest X-ray, we did actually witness development of a large thymus in the first three months post-graft, which is now believed to have played an important role.

These issues will be discussed under the headings of Table I, incorporating the results and experience of the Westminster Team.

I. AVOIDING REJECTION OF DONOR'S OTHER HAPLOTYPE BY HOST

In the past, graft rejection or failure has been observed in up to 50% of aplastic anaemia patients using only a Cyclophosphamide induction (CI), and even occurred in 15% of leukemic patients following total body irradiation (2).

Ia. Suicide Host-Responders to Donor Buffy Coat

Primary antibody responses are recruited in the first 48 hours after exposure to the antigen and such responses can be abrogated by the use of Cyclophosphamide at exactly 24 and 48 hours as long as the dose exceeds 20 mg/Kg. Thus, if buffy coat is given the day before beginning the usual CI, it should help. It has been found that if it is not given at least 24 hours before CI, then what gets killed are the Helper T-cells that are attempting to recruit the other B and T-cells, and it is therefore less effective.

Secondary antibody responses usually occur out of phase and it has been impossible using CI alone to eradicate them. Gorski *et al.* (5) have introduced the novel concept of giving thymic extract to activate Helper T-cells which then will recruit secondary B-cells more in phase; his measured results in mice are spectacular and preliminary attempts in man are encouraging. Thus, where the host might have a secondary response to donor antigens, it seems a good idea to use TP1 (Serono) at 1 mg/Kg with the buffy coat, commencing the CI exactly 24 hours later. Again, because Cyclosporin-A (CYA) itself prevents recruiting IL2 production, it should not be started until the first 2 days of CI are over.

Ib. Cyclosporin-A

This drug acts almost certainly by blocking the Class II receptor OKT4 initiator cells, and therefore must be in that position prior to those cells seeing the new antigen (be it host or donor). Those who have given the drug after that event (for BMT or organ grafts) find themselves committed to life-long CYA, for whenever they stop it, the initial clonal expansion to cytotoxic memory T-lymphocytes can subsequently expand. This seems the best explanation for some late rejections. Markwick *et al.* (13) showed that for either HGV or GVH responses, abrogation could only occur if the drug was present on at least Day -1. There have been problems with what to measure to achieve satisfactory CYA therapy, but if it is the OKT4 receptor that has to be blocked, then surely the free plasma level must be the final arbiter. In vitro studies reveal that a concentration over 100 ng/mℓ is essential to prevent a mixed lympocyte reaction. Whole blood is quite a variable mixture with all the procedures concerned with BMT and we have found that if heparinised blood is collected, delivered at once to the laboratory and held at 37° for 1 hour, and during the separation of the plasma, then the plasma level is reliable (within 8% between batches). As the free level rises above 180—250, the toxic side-effects begin to show and also are cumulative. We admire the use of Ketoconazole by Gluckman *et al.* (4) which perhaps protects the kidney from too much accumulation, making each dose of CYA go further in maintaining a free plasma level; and also, their method for guiding the right dose to rapidly saturate a patient by intravenous therapy at least during the first 14 days (because of the effect of the induction on the gut). Toxicity is worse if sodium is infused in excess, and if the hypertensive effect is not countered by drugs. Finally, we have found that the infusion of a large number of nucleated cells at the time of the graft (or on any subsequent occasion) can rapid-

ly mop up the free plasma level, so that we now add sufficient CYA to such infusions to saturate the contained cells and achieve an adequate free plasma level. With all these precautions, rejection of a donor has been markedly reduced. Of our last 29 half-matched transplants into leukaemics, none has been rejected and among our last 37 other HS patients (without irradiation) only 3 were rejected (see Ic, Id).

Ic. Displacement Induction

Following the method of Santos, but increasing the dose of Busulphan to 80 mg/m^2 for each of four successive days, we have had only one failed take in the last 35 HS transplants; prior to that, 2 girls who had only received Busulphan 2 mg/Kg×4 had rejected their father's marrow, but with the increase in dose, they then accepted their mother's marrow. Today, there is little doubt that intensive induction is necessary to encourage acceptance of HS donor marrow, and with the marked difference seen following irradiation (see II d) we believe displacement induction is the method of choice.

Id. More Marrow Donation

Failure to take has been seen with small marrow doses ($< 2.5 \times 10^8$ nucleated cells/Kg patient weight) and, indeed, twice that dose is preferred or even more if possible. With the many new methods that are being used to deplete T-cells from the donor marrow before infusion, these older guide-lines are no longer valid and each method will have to work out its own marrow dose for the new types of processed nucleated cells. For that reason, we are not yet able to evaluate the one failure mentioned, Ib., but it has been the smallest dose of marrow in our latest series. It is hoped that other workers will keep careful records of their processed marrow dose to guide the future.

Ie. Colony-stimulating Lymphokines

It is now clear that Helper T-cells produce a family of lymphokines which can selectively promote the growth of red blood cells, platelets, phagocytes as well as lymphocytes and, indeed, many other cell lines. The current vogue for depleting the infused marrow of such Helper T-cells, together with the intensive inductions the host will already have received, may well be starving the marrow of such support, and could explain the usual delayed take which is now found. Until such purified lymphokines become available, it seems it might be a good idea to undertake a trial of irradiated donor leukocytes given daily for the first 7 days after the graft. Alas, lymphokines from donor cells might also encourage GvHD, but many centres are still losing patients from infection and bleeding in that first difficult month after the new processed marrow infusions.

II. TOLERISATION OF DONOR CELLS TO THE OTHER HOST HAPLOTYPE

This is absolutely essential to avoid the fatal GvHD which previously was the rule. Currently, the two most popular methods have been the use of CYA or pre-treatment of the donor marrow to remove competent T-cells prior to infusion. The relative success of these two approaches have meant that not enough work has been done in terms of idiotype regulation by the method of autoblast immunisation (9).

IIa. Cyclosporin-A

The introduction of this drug has been responsible for most of the successes with HS grafting, but it is still used badly. It is impressive that the centres at Hammersmith and Paris who have used such extensive monitoring of CYA plasma levels have been achieving the best results with transplants to patients with aplasia. CYA levels should be satisfactorily maintained throughout, and it is sometimes forgotten that only a few days of subnormal levels can allow GvH or HvG clones to develop with either relapse of GvHD or late rejection. Our own Team has experienced 2 late rejections, 1 at 3 months and 1 at 5 months, each associated with inadequate CYA levels because of sharply reduced dosage, perhaps over-reacting to toxic side-effects. At the other extreme, many units will now admit to having killed patients by overdoing CYA because of the breakthrough of GvHD. Long-term CYA has got other serious side-effects, and it does appear that as it binds to the kidney, the cumulative effect can become irreversible. What is badly needed is some kind of assay to tell us that it is safe to stop CYA.

IIb. Removal of T-cells Before Marrow Infusion

Following work in F1-Hybrid situations in experimental animals, polyclonal rabbit anti-

human lymphocyte sera were prepared in Munich and initially used with success in preventing GvHD (17). This was an extension on the concept of Hobbs *et al.* (8) that the early removal of those lymphocytes which were competent to respond against the host, might allow their successors to emerge more slowly and become tolerised to that host. Subsequently, other batches of rabbit antisera did not produce such good results. These difficulties continue, but with the introduction of monoclonal antibody production, it has been possible to prepare better standardised products, often cocktails, because of the relative weakness of a single monoclonal antibody. Other ways of reinforcing such antibodies have been the addition of cytotoxic agents to them. Others have followed the original approach of Dicke, initially in Van Bekkum's laboratory, to remove such T-cells by other fractionation methods. This work is reviewed elsewhere in this Symposium, but in the tough situation of the HS donor, 3 different methods recently reviewed in Fillerval (INSERM, 1984) had eventually only produced about 50% of good survival which is about the same as that which can be obtained with CYA, properly used. It follows that further improvements are more likely by combining IIa and IIb, as we are now doing.

IIc. Autoblast Immunisation

The Westminster team have undertaken 2 transplants from HS donors who have undergone autoblast immunisation in the 3 weeks before the transplant. In neither case was the MLC totally abolished, and while each of the patients did develop some GvHD with some chronicity, both have now completely recovered. This promising area (9) seems worthy of further exploration: it has the disadvantage at present, that up to one-third of the donors, or indeed, the recipient will have increased sensitisation as a result of the attempt, excluding the possibility of their being used. Furthermore, it should not be used directly for leukemic patients for fear that although the donor cells are autoblasted in vitro, their subsequent inoculation could carry over leukemic antigen or, indeed, agent. It would be a disaster to tolerise the donor to the latter and, of course, might prevent IV. If there was enough time, it would be a feasible idea to use one parent with the other Haplotype to try and tolerise the other parent; in this way, the future donor if achieved, as can be assessed by the MLC, would not have been tolerised to the child's leukemia. To be able to undertake a transplant for leukemia with no other immunosuppression would give the best possible chance for IV to happen.

IId. Non-Irradiation of the Thymus

While thymic epithelium can survive 5 GY, it functions poorly after 10 GY, and the evidence of reduced thymic hormone levels is accumulating to add to the known long-lasting lymphopaenia of many cancer patients whose thymus was in or near the target field. Before and after birth, it is now clear that large numbers of emerging lymphocytes visit the thymus, which appears to be rich in host antigens so that the T-cells that then leave the thymus have been selected for non-reactivity. Alas, in the adults it appears that only about 1.5% of the bone marrow precursors pass through the thymus each day. This may explain why in young children, tolerisation seems to have been easier to obtain, as the flow is almost certainly higher. There is, however, evidence that T-cell depletion (by chemical induction, etc.) can result in increased thymic activity with raised levels of thymic hormones. Animal studies have shown that the full benefit of CYA is not achieved if the thymus is ablated or irradiated in an F1-Hybrid situation. Accordingly, we have tested our data where the patients have been evaluable by having shown engraftment and surviving long enough for such processes to occur. The results are shown in Table II where, despite the same attack rate for GvH of Grade 2 or higher, (at 64%), the death rate attributed to GvHD was over twice as high for the irradiated patients. The eventual survival shows marked differences and in another study of leukemic patients, only 3 of 35 were reported as surviving beyond 2 years (16). The GvH has been so much more severe, that intensive immunosuppression with high-dose corticosteroids and other drugs has been necessary and, doubtless, this by impairing IV has contributed to the high relapse rate for leukemia which accounts for many of the other deaths (in Table II, although the relapse may have been due to the GvHD, it has not been attributed to it).

Since most of our patients have been children and are comparable in age and sex, we feel justified in concluding that the thymus is probably quite important in achieving decent survival from an HS transplant and it can be stated that all 9 of our non-irradiated patients who have survived beyond 2 years, have a good life, and appear to be in a state of active tolerance. It has to be admitted, however, that this marked difference between irradiated and non-irradiated subjects may be due to some other, as yet, unrecognised factors.

IIe. The Need for an Assay of Tolerance

HS transplants are often followed by prolonged use of immunosuppressive treatments, especially the use of CYA. How long adequate CYA levels need to be maintained varies for different forms of transplantation, is still being elucidated, and remains a major problem area at the present time. In some patients the drug has had to be stopped because of its unwanted effects, and either a hard-won graft has been lost or GvHD has occurred. On the other side, continuing the CYA for prolonged periods pays another price in preventing responses to new infectious agents or emerging lymphoma etc. (12). There is an urgent need for better assays to indicate when tolerance may have been achieved. Because CYA itself probably blocks clonal expansion selectively for OKT4, the numbers of these cells or their ratio compared to OKT8 are invalid while CYA is being continued at adequate levels. CYA does not achieve tolerance by deleting responsive clones (13), but by holding down OKT4 receptors, permits the development of OKT8 regulatory suppressor responses. Recent work (6, 7) suggests that transplanters should store in liquid nitrogen pre-graft responder cells (donor or recipient) and the immunogenic cells of the graft. It would then be possible to test the peripheral blood lymphocytes of the post-graft patient before and after stripping out the nylon wool-adherent suppressor cells to see whether or not there is suppression of any pre-graft responses. If the post-graft cells were able to suppress an initially positive response, and this was abolished by nylon wool, such an assay might indicate the emergence of specific suppressor cells. To perform these tests it would be necessary for the pre-graft samples to be stored without any immunosuppressives, and the post-graft samples would need to be washed free of them. A similar kind of assay needs to be developed for other approaches to tolerisation.

III. IMMUNE COOPERATION

In our hands, normal HS leucocytes have cooperated better with immunodeficient leukocytes than have outbred ones (9). The long survival of 9 of our patients who received HS grafts has not been complicated by any serious viral, fungal or mycobacterial infections, indicating that in practice one genetic haplotype permits adequate donor lymphocyte regulation of host intracellular infections.

Evidence has been published (9, 10) suggesting that MLC negative phenotypically-identical adults may not adequately protect a host.

The work of Touraine *et al.* (18) shows that HLA-restriction is not absolute when syngeneic foetal liver and thymus (from under 12 weeks gestation) has been allowed to emerge slowly within a clean host.

In general, however, the Zinkernagel (20) hypothesis could be tested in advance of some transplants (not emergency) to check that the prospective donor leukocytes could, in vitro, deal with host fibroblasts infected with virus in culture conditions. Similarly, if a stock of host fibroblasts were kept, it would be possible to undertake further assays post-graft to see if immune cooperation is continuing or being influenced by therapy.

It is very important to control the herpes group of viruses, which so easily break out post-graft. Via Interferon induction they increase HLA and DR expression by tissue cells, as it were accentuating the differences at a time when developing tolerance might be better assisted by no such activity.

IV. IMMUNE ELIMINATION OF RESIDUAL NEOPLASIA

The widespread use of BMT to treat leukemia and now a growing number of neoplasms (neuroblastoma, melanoma, lymphoma, myeloma, etc.) has suffered too long from the misconception that even modern chemotheraphy or radiation treatment can itself eliminate the last tumour cell. There is evidence that allogeneic donors often recognise

the tumour cells as distinct from the host's other cells (11) and that immune elimination corrects the minimal residual tumour state (3).

The measures currently used to enable HS engraftment without GvHD all counter such immune elimination. CYA abrogates primary immune responses; removal of competent T-cells from donor marrow allows residual tumour cells to gain a lead, possibly into an antigen-excess situation over newly emerging donor clones and high-dose corticosteroids, azathioprine, methotrexate will similarly impair immune responses. Perhaps this is why modern relapse rates following transplants for leukemia are so high, but especially so if only those surviving HS grafts long enough to be assessed are counted. We are not yet aware of a 4-year disease-free survivor following an HS graft.

Furthermore, it is known that the long-continued use of drugs such as CYA and azathioprine are associated with development of secondary neoplasms (12).

V. SELECTION FOR HS TRANSPLANTS

Va. Which disease?

The previous record in aplasia and leukemia is depressing (11, 15). Perhaps, the evidence in Table II should encourage exploration of HS grafting in immunodeficiency and other inborn error situations before renewing the attack on leukemia. The initial results seem more cost-effective and would allow the various methodologies to be better worked out without the problems of IV. Perhaps the younger patients also permit better studies of II.

Vb. Which donors?

Because of the difficulties following irradiation induction, it is worth looking at the Westminster results for non-irradiated transplants (see Table III).

The HS siblings seem more likely to have the closest genotype match, their DNA representing post-selection expression of the parenteral contributions. 'Other HS relatives' combines data on parents with uncles, aunts and cousins etc. because, as yet, no significant difference has been observed. As our numbers grow, we will check again, especially uncles. To date, HS transplants from 15 fathers have produced 5 rejections, 4 deaths from acute GvHD alone, and 3 deaths from infection, possibly permitted by the additional immunosuppression required to control GvHD. Only 3 survive alive and well. From 5 mothers, all grafts have produced severe, chronic GvHD with one death from a "stiff-man" syndrome, although by 4 years one patient seems to have finally become well and able to lead a normal life. It is as if mothers have been sensitised by their pregnancy, although this could not be shown by Moore *et al.*, (14). Similarly, it is possible fathers have been sensitised to the mother's haplotypes by repeated mating. Our order of choice for HS donors would therefore be:

1) Sibling
2) Other non-parental relative
3) Father
4) Mother.

It has not been possible to yet fully analyse the relevance of the degree of positivity of the MLC, but we have the impression that the lower increments more rapidly appear to achieve tolerisation.

ACKNOWLEDGEMENTS

Essential help has come from many other members of the team. With these difficult transplants we are especially grateful to Drs. L. Ingram and S. Desai; microbiologists T.R. Rogers, S. White and M. Petrou; Sisters Gale Sims, Sarah Needham and Bridget Beilby and psychologist Ms. Coreen Pot. Thanks are also due for the financial support of the Bostic, Fane, Dobson and Nolan trusts.

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Table I Objectives using donors sharing one genetic haplotype.

I. AVOID REJECTION OF DONOR'S OTHER HAPLOTYPE BY HOST
   a. Suicide host responders to donor buffy coat.
   b. Cyclosporin-A.
   c. Displacement induction.
   d. More marrow donation.
   e. Colony-stimulating lymphokines.

II. TOLERISATION OF DONOR TO OTHER HOST HAPLOTYPE
   a. Cyclosporin-A
   b. Removal of T-cells before marrow infusion.
   c. Autoblast immunisation.
   d. Non-irradiation of the thymus.
   e. The need for an assay of tolerance.

III. IMMUNE COOPERATION BETWEEN DONOR AND HOST

IV. ? IMMUNE ELIMINATION OF RESIDUAL NEOPLASIA

V. SELECTION FOR HS TRANSPLANTS
   a. Which diseases?
   b. Which donors?

Table II To show the effect of irradiation on the outcome of BMT from HS donors. Death rate refers to those attributable to the GvHD itself.

PATIENTS EVALUABLE	TOTAL BODY IRRADIATION 10 Gy OVER 4 HOURS	GvHD GRADE 2 or> ATTACK RATE	DEATH RATE	SURVIVAL CRUDE	OVER 2 YEARS
14	YES	64%	57%	21%	1/14
33	NO	64%	27%	45%	9/33

Table III Choice of HS donor. Results of elective transplants, not using irradiation for induction, by an experienced team (Westminster).

PATIENTS EVALUABLE		SURVIVAL	ORDER OF CHOICE
	MLC-NEGATIVE (TI<1.84)		
24	MATCHED SIBLING	100%	1
11	SIMILAR RELATIVE	55%	3
	MLC-POSITIVE		
12	HAPLOTYPE-SHARING SIBLING	67%	2
24	HAPLOTYPE-SHARING RELATIVE	50%	4