Prevention of Graft versus Host Disease Following Allogeneic Bone Marrow Transplantation


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INTRODUCTION
It is almost 20 years since allogeneic bone marrow transplantation (BMT) became a reality in man, and the first attempts at controlling graft versus host disease (GvHD). This consisted of the use of methotrexate given after transplantation and was based upon empirical studies in dogs (12, 13), in which controlled trials had shown that methotrexate was preferable to no treatment for both reducing the incidence of the syndrome and improving survival. Unfortunately, it was not ethical to undertake a trial in man comparing methotrexate with no treatment and so early studies were almost entirely confined to patients receiving methotrexate after transplantation, and this was usually given for approximately 3 months (14). In 1978 the immunosuppressive agent, cyclosporin A (CyA) became available, and was shown to be effective in the treatment of skin graft versus host disease (8). Subsequently, when used prophylactically after transplantation CyA was found to decrease the incidence of fatal graft versus host to 5% although transient graft versus host disease continued to occur in approximately 70% of patients (9). In this paper we will discuss our more recent results using cyclosporin A for matched transplants and compare these with a pilot study of the use of a monoclonal antibody to purge the marrow of immunocompetent mature cells.

CYCLOSPORIN A WITHOUT OTHER IMMUNOSUPPRESSION AS PROPHYLAXIS AGAINST GRAFT VERSUS HOST DISEASE

Seventy-two patients with AML, received BMT during first remission at the Royal Marsden Hospital. The age range was 4–46 years with a median of 37 years. Fifty-six patients received, as conditioning, cyclophosphamide 60 mg/kg i.v. daily for 2 days followed after 96 hours by total body irradiation (TBI) of 10 Gy given as a single dose at a dose rate of approximately 1.5 Gy/h. An additional 11 patients were conditioned with a priming intravenous dose of cyclophosphamide 300 mg/m² followed 7 days later by a single i.v. dose of melphalan (85-100 mg/m²) and 12 hours later TBI at a dose of 10 Gy. Both these groups of patients received the marrow transplant the day after the TBI. A third group of 5 patients received the same priming dose of cyclophosphamide, followed 7 days later by a single dose of melphalan 240 mg/m² (and no TBI) and 4 days after the melphalan they received their transplant.

Approximately 2.0 – 3.5 × 10⁸/kg nucleated bone marrow cells were given from HLA/DR matched sibling donors. The marrow was not subjected to any separation procedures prior to infusion. Five different cyclosporin A regimens were used as GvHD prophylaxis. Thirty-eight patients received intramuscular CyA 25 mg/kg from the day prior to transplant for 5 days followed by oral CyA 12.5 mg/kg for 175 days. An additional 5 groups of patients received oral instead of i.m. regimens as follows; from the day prior to transplant CyA was given for 5 days at a daily dose of either 8 mg/kg (7 patients), 12.5 mg/kg (15 patients), or 55.5 mg/kg (8 patients) followed by a maintenance dose of 8 – 12.5 mg/kg for 175 days. In all studies the maintenance dose of CyA was titrated against toxicity with the dose being reduced by 33% if the creatinine rose above 250 μ mol/l or the
BUN was above 20 mmol/l. When i.m. CyA was no longer available four patients received 12.5–25 mg/kg/day infused over 12 hours for the first 5 days of transplant.

Initially all patients developing skin rashes had a biopsy taken, but no change was made in management and in all but one instance there was complete resolution of the skin problem. After our first fatality with GvHD all patients with skin GvHD received as treatment 3 days of i.v. methylprednisolone 700 mg/m². Recently skin biopsies have no longer been performed.

RESULTS

Of the 38 patients receiving i.m. CyA 26 (68%) are alive; 71% either had proven or suspected GvHD and one (3%) died of the syndrome. There was no significant difference in the results obtained for the 30 patients treated only with oral CyA; 56% are alive; 66% developed suspected or proven GvHD and one (3%) died from it.

Forty-seven of the 72 patients remain alive with a projected actuarial plateau between 26 and 56 months at 54%. Of the 25 deaths, 8 (11%) were due to recurrent leukaemia (day 92–393; median 189) 6 due to pulmonary oedema (day 30–513; median 92), four due to renal failure (day 27–84), 3 pneumonia (day 41–65) 2 GvHD (day 49, day 77) and 1 CNS (day 20) and 1 carcinoma of the rectum (day 698). There were thus 23% transplant related deaths. Four of the 8 patients receiving high initial CyA doses (55.5 mg/kg/day) died of pulmonary oedema whereas there were only 2 deaths from this cause in the whole of the rest of the series. Otherwise the causes of death could not be related to the CyA administration. Although renal dysfunction occurred in over 70% of patients on CyA, death due to renal failure was not directly attributable to CyA. However, a correlation was found between the conditioning regimen and severe renal failure; only 1 patient died of renal failure of 56 conditioned with cyclophosphamide and TBI, whereas 3 of 16 patients who received melphalan (with or without TBI) died of the problem.

Age was the most important factor associated with survival; of 23 patients under 20 years of age 80% remain alive; of 18 patients between 21 and 30 years 67% are alive; of 24 patients between 31 and 40 years 59% are alive and only 43% are alive of 7 patients over 40 years of age.

Forty patients stopped taking CyA after 180 days and 12 required reinstitution of the drug within 60 days due to the development of acute GvHD in either skin or liver (based on liver function tests). All had resolution of their GvHD.

It was gratifying in this present series to find that only 4% of patients died as a direct consequence of GvHD. In this series of transplant patients there was still a relatively high proportion (23%) of deaths due to causes other than leukaemia. Fatal pulmonary oedema (a problem which dominated our mismatch transplant programme (10)) was the most common serious occurrence and seems almost certainly due to increased vascular permeability related to the administration of CyA although no direct correlation could be found between the dose of CyA, the serum levels of CyA and this complication (1). Increased vascular permeability probably also occurs in the brain in patients on CyA and several of the younger patients had convulsions during the first 4 weeks after transplant. It is of major concern that drugs (including cyclosporin) which do not usually cross the blood-brain barrier may be capable of leaking into the cerebral parenchymal tissue when vascular endothelial permeability is increased and this could lead to CNS toxicity of drugs that would otherwise not affect the brain. We think this happens in patients on CyA. It has been known for some time (4) that CyA alters vascular permeability in patients with SLE.

Renal failure leading to death, the next most common serious complication, seemed to have a separate pathogenesis from the mild renal toxicity seen in most patients on CyA (1, 3, 8) in that severe intravascular endothelial lesion is probably the underlying cause (5). Further studies are required to determine its exact pathogenesis.

PURGING OF BONE MARROW WITHOUT OTHER IMMUNOSUPPRESSION AS PROPHYLAXIS AGAINST GRAFT VERSUS HOST DISEASE

Bone marrow transplant using cyclosporin A is now producing a cure rate of approximately
80% in children with acute myeloblastic leukaemia in first remission (15). However, it is quite clear that there is a toxicity to cyclosporin A which is still producing morbidity and which is significant. In older age groups this problem is much greater. As a consequence, we and others have explored the possibility giving the patient no immunosuppression other than the bone marrow purged of immunocompetent mature cells prior to transplant. The rationale of this is that stem cells remain in the purged marrow and are able to reconstitute a normal immune system and these cells are able to proliferate in the host in a background of immunological tolerance. This may be comparable to the events that occur in neonatal life.

There are many methods of purging marrow of immunocompetent cells and this includes mechanical methods such as soybean agglutination (7) and a variety of xenogeneic antisera. However, with the advent of monoclonal antibodies directed against specific cell populations a more scientific and rational approach to the purging process has developed. Other groups have studied a variety of monoclonal antibody cocktails directed against specific cell populations (6, 11). In our pilot study reported here we have used a rat IgM monoclonal antibody CAMPATH I that avidly binds human complement and has a broad spectrum of activity against mature immunocompetent cells including mature T-lymphocytes, B-lymphocytes and probably monocytes and macrophages (2). It does not have any lytic action against any of the colony forming cells of the myeloid system found in human marrow.

Three methods of using CAMPATH I have been used. In the first instance we have used a Ficoll metrizoate gradient with the IBM 2991 blood cell washer, which produces a mononuclear cell layer. CAMPATH I was added to this cell population, the cell suspension was washed once and without the addition of human complement the cells were infused and tests showed that in-vivo lysis occurred. The second method involved the separation of a bone marrow buffy coat using hetastarch to which human complement was added in addition to CAMPATH I and the remaining cells were infused after a single washing. The third approach was to obtain a buffy coat using the IBM 2991 blood cell separator; CAMPATH I and human complement were then added and again after washing the nucleated cells were infused into the recipient. The yield of cells using these three methods will be described below.

RESULTS

Nineteen patients have received bone marrow transplants with CAMPATH I purging of donor marrow. Four of these were autologous and 15 allogeneic. The mean age of the group is 22.5 years, 11 were male and 8 were female. Of the 15 patients receiving allogeneic transplants 12 received HLA matched grafts and the other 3 received marrow from HLA mismatched family donors. The overall survival of the whole group is 68% (13/19). Of the 15 patients receiving allogeneic grafts 10 remain alive (matched 8/12, mismatched 2/3). The number of cells infused using the three different methods is as follows: 10 patients received the Ficoll metrizoate treated marrow at a mean infusion dose to the donor of $0.7 \times 10^8$/kg (range 0.28–1.8); 6 patients received the hetastarch treated marrow at a mean infusion dose of $1.09 \times 10^8$/kg (range 0.64–1.58) and three patients received the IBM 2991 separated buffy coat at a mean infusion dose of $3.4 \times 10^8$/kg (range 3.0–3.8).

The effects of the three different methods of separation on infused CFC-GM/kg body weight is as follows; Ficoll metrizoate method $7.1 \times 10^4$, hetastarch method $9.4 \times 10^4$ and IBM buffy coat method $22.4 \times 10^4$.

ENGRAFTMENT

Of the 12 HLA matched transplants 6 received marrow using the Ficoll metrizoate method and 3 required regrafting. Of the 6 who received the hetastarch or buffy coat marrow, all had prompt engraftment. The mean time for patients to acquire $0.5 \times 10^9$/? polymorphonuclear leukocytes in the peripheral blood was 44 days for the Ficoll metrizoate group of patients (excluding those who required retransplantation) and 20 days for the patients receiving the hetastarch and buffy coat marrow.

FIRST REMISSION AML

Nine patients with AML in first remission received allogeneic matched transplants in this study. Five received the mononuclear treated marrow (Ficoll metrizoate method) and 4
received the buffy coat marrow (hetastarch method and IBM 2991 separated method). Of the 5 receiving the mononuclear cell layer 3 required a second transplant due to failure of graft take, and 2 of the 3 have subsequently died. Of the 4 patients who received buffy coat cells all had prompt engraftment and are alive and well.

**GRAFT VERSUS HOST DISEASE**

In the whole series of 15 patients graft versus host disease only occurred in one patient. This patient was retransplanted following failure to engraft with untreated marrow and cyclosporin A. There had been no evidence of GvHD after the first transplant.

Five of the 15 patients who had allogeneic transplants have died, 4 due directly to failure to engraft, and the fifth patient died of GvHD but required retransplantation due to initial graft failure. Although these results are preliminary and the longest survivor is only at 212 days, there nevertheless is an indication that CAMPATH I treated marrow, in which a buffy coat method is used allows for prompt engraftment without graft versus host disease. These results warrant a controlled trial comparing cyclosporin A with CAMPATH I purged marrow. The important questions to be asked in addition to overall survival and graft versus host disease is whether there is a difference in death due to bone marrow failure, duration of stay in hospital with cost implications, and the incidence of recurrent leukaemia in the two groups. This latter point is related to a possible antitumour action of an immunocompetent graft. Age will undoubtedly be an important factor. It is likely that the very high success rate in children receiving matched transplants with cyclosporin A will not be superceded by purged marrow. However, morbidity may be reduced in this group. In older patients where cyclosporin A appears to be more toxic, it may well be that purged marrow will be a preferable form of treatment with benefit not only in terms of morbidity but also survival.

In this report we have not discussed the results of mismatched transplants because the numbers are too few. However, it seems likely that success in mismatched transplants is going to depend upon a combination of purged marrow and the use of cyclosporin, with cyclosporin being used not only for its effect on graft versus host disease, but also for its immunosuppressive qualities in allowing engraftment to occur.

The much faster and more complete engraftment that we have seen using the buffy coat method as compared to the mononuclear preparation seems most likely to be related to immunological rejection of the graft rather than the removal of essential bone marrow stromal cells by the CAMPATH I. The buffy coat method of treating the marrow allows for a much higher inoculum of cells which may result in antigenic excess swamping any remaining immunity in the irradiated recipient. However, it is possible that additional immunosuppression to the recipient using drugs such as Ara-C may allow engraftment of marrow using mononuclear cell preparations. This is clearly an important area of research in the future.

**REFERENCES**

8) Powles RL, Barrett AJ, Clink HM, Kay HEM:
15) Unpublished data. The Royal Marsden Hospital Transplant Group. 1984
Storb: Any questions for Dr. Goss?
Pollard: Have you no problems with cytomegalovirus?
Goss: We've had a very low incidence of cytomegalovirus and in the last 6 months, we have only seen 3 patients with cytomegalovirus and we have a very good lab at St. George's Hospital where they are screened through.
Storb: Dr. Ernst, you had a question.
Ernst: Yes. I have heard his presentation several times, and I wonder, this capillary leak syndrome, how frequent is it actually, because I think that the Royal Marsden people have a very much higher frequency of this syndrome than, for instance, in Copenhagen and Seattle. So I really wonder world-wide, how frequent is the symptom.
Goss: I don't know what the world-wide incidence is. It's not all that frequent in our matched program. But it certainly is frequent in our mismatched program.
Storb: Dr. Gratwohl?
Gratwohl: I think there is one possible explanation to it. Your incidence in graft vs. host disease is very low compared to our incidence. But I'm sure that if I would be at the Royal Marsden, and you would be in Basle, we would see the same difference because we do not know at the moment what is GvHD in cyclosporine-treated patients. And I, for my part, am convinced that this pulmonary and this endothelial story is one aspect of graft vs. host disease. We know that endothelial cells can be a target of graft vs. host disease and that's just one part of the story. It might well be a problem of interpretation.
Goss: Certainly it may be lung involvement in graft vs. host disease. However, it's not classical interstitial pneumonia because what you see is a hemorrhagic pulmonary edema without the pathological interstitial changes normally described. So it's very different from interstitial pneumonia, but it may be graft vs. host disease.
Storb: I think there is one best possible explanation. First of all I don't believe in lung graft vs. host disease. But that's my opinion. There's no real proof for that actually in any experimental animal. But one of the problems that you have, perhaps, or one of the reasons for your capillary leak problem is your very high doses of cyclosporine you're giving, at least compared to us. I think you're giving about 2 to 3 times the dose that we would be giving in a similar patient, and that may be the reason why you see more capillary leak problems.
Goss: Yes. That is the reason why we've cut back on our cyclosporine dose dramatically now.
Storb: You have.
Goss: Yes. This was up until recently. There's a certain amount of pathological evidence that this capillary leak syndrome actually exists in that first of all in fluorescein dye studies of the eye, we've demonstrated in giving patients intravenous fluorescein, we've been able to pick up—ophthalmologists have been able to pick up—fluorescein leaks in the retina. We've also seen bilirubin-staining of the brain at postmortem. And in normal adults, bilirubin doesn't cross the blood brain barrier, and the other peculiar abnormalities which...
Storb: You may find that by reducing the dose, you'll see less of it. I think there's room for one more question. Dr. O'Reilly you had something to say, or Dr. Hobbs?
Hobbs: I was only going to make one comment, and that is that we used exactly the same irradiation on 60 of our patients and we only saw the syndrome in 2. And the reason is that the 2 of them were much more susceptible to CS-A and we can't allow them to die of renal failure. But we had seen the symptoms, in children who were CS-LE who is not high, and we do accept that there is such a thing and I do believe that there probably is higher, but never be comparable with GvHD.
Storb: We move onto the next issue, just to stay on time. Perhaps there's some time to pick up this discussion at the end where we'll have another 10 minutes. Now we go onto Dr. Masaoka who is going to speak about bone marrow transplantation in Japan. Dr. Masaoka.