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ALLOGENEIC BONE MARROW TRANSPLANTATION

Recent developments and the potential expansion of the donor pool

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Abstract

Graft versus host disease is a preventable complication of allogeneic bone marrow transplantation. A complex biological manoeuvre has enabled us to achieve this. Unfortunately T-cell depletion of the donor marrow shifts the delicate immunological balance in favour of the recipient immune system. The consequence has been an increased risk of graft rejection and leukaemia relapse. It is necessary to shift this balance back in favour of the donor (derived) immune system. This can be achieved by increasing the immunosuppressive power of the 'conditioning' chemoradiotherapy. Toxicity considerations limit our scope although single fraction fast dose rate radiotherapy appears to diminish the risks inherent with T-cell depletion. Logically an approach with greater promise will be to use specific immunological means of eliminating the recipient T lymphocytes in vivo. Early experience with a monoclonal anti-lymphoid antibody in vivo in an HLA matched unrelated donor programme suggests that this approach has great promise whilst lacking toxicity. We hypothesise that a shift in immunological superiority to the donor derived immune system will not only allow T-cell depletion whilst minimising the risk of graft rejection, but also enhance the anti-leukaemic properties of the graft. Of further interest will be the potential for application of lymphokines (e.g. IL2) without increasing the risk of lethal graft versus host disease.

Key words: Leukaemia, bone marrow transplantation, T-cell depletion.

The treatment of leukaemia by bone marrow transplantation (BMT) (28) was originally thought of as a means of rescue from marrow ablative ('supra-lethal') radiotherapy or chemoradiotherapy. This concept has been somewhat modified by the observation of an apparent graft versus leukaemia (GvL) effect (31). It is our contention that GvL may, in fact, be paramount in the eradication of the disease.

T-cell depletion of donor marrow was introduced as a means of preventing graft versus host disease (GvHD) and has led to the near total elimination of this problem but has not, as yet, led to a corresponding increase in survival. The 'quid pro quo' for the prevention of mortality due to GvHD has been an increased risk of graft rejection and in some studies an increased risk of leukaemic relapse. Here we describe the technology and clinical results of T-cell depletion and deal with the lessons learned from these studies and describe how they point the way ahead in the application of the various cytokines in BMT and to the expansion of the donor pool in allogeneic BMT.

Methods of T-cell depletion. The original technique used in pre-clinical studies was that of physical separation using discontinuous albumin gradients (6) which exploited the different densities of lymphocytes and marrow progenitors. Although other physical methods, such as elutriation (5), are used in clinical practice most teams now employ immunological methods in which conventional (rabbit) anti-T sera (25) have given way to the more selective and more readily produced monoclonal antibodies (MAbs) (14). Our original studies with MAbs included the use of the pan-T antibody OKT3 (CD3) in which the coated target cells within the donor marrow were reinfused into the recipient (7).

More recently many groups, including our own, have used anti-human T antibodies which are lytic in the presence of rabbit (12, 23) or human (30) sources of complement (C'). Other groups are studying the use of antibody coated magnetic microspheres (29), toxin conjugated anti-T antibodies (8) soybean agglutinin and sheep red blood (E) cell rosetting (24). It is our contention that in HLA matched allogeneic BMT there is little to choose between these methods; all reliably achieve close to 2 logs depletion, but the E rosetting method may be preferred when a

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higher degree of depletion (3–4 logs) is required, as in the HLA mismatched setting.

Bone marrow engraftment and host versus graft (HvG). Although graft rejection (HLA matched) was a problem in BMT for severe aplastic anaemia (27) it was seen only infrequently (approx. 1%) as a complication of BMT for the treatment of acute leukaemia using conventional immunosuppression (IS) for prevention of GvHD. With the introduction of T lymphocyte depletion some groups saw a dramatic increase in the risk of non-engraftment or engraftment followed by graft failure (17, 19). Graft rejection was most frequently seen around 4–6 weeks but could be seen several months later. An analysis by our collaborative group (19) suggested that the risk of rejection was inversely related to the intensity of the conditioning regimen used. The scheduling and dose of irradiation are particularly critical. The use of fractionated total body irradiation (F-TBI) in a total dose of 10–12 Gy delivered in 2 Gy fractions was associated with graft loss in 8/13 cases. In contrast only 1/38 patients receiving (a planned) 7.5 Gy in a single fraction at a fast dose rate (0.26 Gy/min in air) rejected their graft. Subsequent studies suggested that where the total dose of TBI in the single fraction regimen fell below 7 Gy then the risk was again enormously increased.

It seems that intensified radiation conditioning can reduce the risk of HvG problems but that the therapeutic window is narrow. Several groups have explored the use of more intensified chemotherapy conditioning without apparent benefit. Immunological techniques using monoclonal antibodies to treat the recipient may be more promising (4). Our own group now has experience in the use of immunological conditioning in HLA mismatched and, more recently, HLA matched unrelated donor T depleted BMTs: the latter are proving particularly promising with no rejections yet seen in 7 consecutive BMTs.

Graft versus host disease. The risk of significant (≥Gd. II) acute GvHD has been dramatically reduced by even modest (90–99%) T-cell depletion (7, 22, 23) and with 2–4 log depletion the problem of acute and chronic GvHD is abolished (18) even in HLA mismatched BMTs. In HLA matched BMT significant acute GvHD is infrequent providing less than 1×10^6 T lymphocytes per kg recipient weight are infused (13).

Immune reconstitution. The pessimists predicted long-term immune deficiency in the recipients of T lymphocyte depleted BMT. In fact such paresis is more likely to be seen in conventional (immunosuppressed) recipients (35).

We have demonstrated a relatively slow recovery of T helper (CD4 +ve) populations in recipients of T lymphocyte depleted BMT compared with conventional IS but a lack of the characteristic T cytotoxic/suppressor overshoot. As a result the T4:8 ratio was less disturbed (>0.4:1) in the recipients of T depleted BMT (7, 13). The next, surprising, observation we made was that in our patients (who since 1983 have received no post-transplant immunosuppressive drugs) the immunoglobulin isotype levels remained within the normal range (3).

We next studied the adoptive transfer of B cell immunity from donor to recipient. We found that immunisation of *donor* (D') pre-transplant led to a shortlived specific antibody (Ab) response in the *recipient* 3–5 weeks after the transplant. Where both donor (D') and recipient (R') were vaccinated then the response was increased in titre and duration by about 1 log. This presumably is a result of further antigenic 'presentation' in the host (33). Additionally 3 months after BMT we found that this Ab response could be boosted by repeat immunisation only in the D'R' group (34). This response may be of more than theoretical interest since we have recently observed that a cytomegalovirus (CMV) immune donor confers significant protection to a recipient who is also seropositive for CMV (10). CMV reactivation is one of the major obstacles to success in allogeneic BMT mainly because of the complication of CMV pneumonitis (32). The availability of an effective (probably recombinant) CMV vaccine is awaited.

Given that we have observed low levels of T helper (CD4 +ve) lymphocytes at the time of these specific antibody responses, we set about searching for the source of 'help'. At the time antibody titres peak we have observed a wave of mononuclear cells appearing in the blood, a high proportion of which have the morphology of large granular lymphocytes (LGL). Isolation and study of these cells show that they have a pattern of target cell killing characteristic of 'activated' natural killer (NK) cells. They spontaneously kill not only the classical NK target K562 but also T-cell lines and EBV positive cell lines (26) (which may be important in protecting these patients against EBV infections and lymphomas). Supernatants from these cultured LGLs proved capable of driving immunoglobulin production in vitro and were shown to contain B cell differentiation factor (BCDF or IL6) as well as other lymphokines including Interleukin 2 (IL2). We postulate that these factors may be one source of help for the B cells (2).

These cells are also capable of killing host (cryopreserved) acute myeloblastic leukaemia (AML) blasts (15) (see below).

In vitro the activity of these LGLs against all targets tested can be boosted by the addition of recombinant (r) IL2 which further enhances secretion of other cytokines (15).

Leukaemia relapse. Following the introduction of T-cell depletion, some groups have observed an (apparent) increase in the relapse rate of leukaemia. This has been seen in chronic granulocytic leukaemia (CGL) (1) and acute myeloblastic leukaemia (20). Preliminary analysis of the European Bone Marrow Transplant Registry (EBMT) (21) supports the observation of an increase in CGL but, in contrast, if T-cell depletion alone (i.e. no immunosuppression) is used then there is no apparent increased risk in acute leukaemia transplanted in first CR.

In concurrence with the EBMT analysis, our own group has seen no increase in relapse rates in acute leukaemia. Preliminary observations by the Campath-1 users group (11) and the EBMT (21) in patients with CGL transplanted in first chronic phase suggest that 2 factors may play a role. First, the use of fractionated radiotherapy in conditioning is associated with a trend to a higher relapse rate in T-cell depleted BMT (EBMT not significant, Campath $p=0.04$) and, second, the use of post-transplant immunosuppression (EBMT not significant) is also associated with a higher rate of relapse. The strongest correlation in the Campath analysis was an increased relapse risk with slower engraftment. Further analysis of these and other parameters will be required before firm conclusions can be drawn.

The interpretation of these observations varies. The Campath group prefer the hypothesis of a *physical* graft advantage for the donor marrow where non-fractionated radiotherapy is used. We prefer the possibility that both the use of single dose TBI, which is more immunosuppressive than equivalent fractionated regimens, and the avoidance of post-transplant immunosuppressive drugs confer an *immunological advantage* to the donor marrow. The latter concept is supported by our observations of a lower rejection rate with single fraction TBI.

Perhaps more important is the effect on the LGLs (see above) of post-transplant I. S. Our preliminary observations suggest a low level of activation of the LGLs if the patient is on immunosuppressive drugs and this can be enhanced by their withdrawal. Another intriguing observation has been made by the group from Genova. Some patients who received a T lymphocyte depleted BMT for the treatment of CGL were found to have a cytogenetic relapse post-transplant (i.e. Ph' +ve). Withdrawal of cyclosporin treatment resulted in reversion to a normal cytogenetic pattern in a proportion of the patients (9).

Our hypothesis is that the 'conditioning' of the recipient plays a relatively minor role in the cure of the leukaemia and that the disregulated donor derived immune reconstitution, with high levels of activated LGLs and perhaps also the reversed T4:8 ratio play a major role in elimination of minimal residual disease. If this hypothesis is correct then in T-cell depleted BMT where the cytotoxic T lymphocyte effector of GvL is largely lost the elimination of residual leukaemia is almost totally dependent upon the LGL population.

Application of lymphokines in bone marrow transplantation. With the long-term aim of boosting the anti-leukaemic activity of the LGLs after allogeneic BMT we have undertaken a series of studies to establish the optimal therapeutic/safety scheduling of the use, in vivo, of IL2. Our studies in a mouse model suggest that if we are to use IL2 in the clinic then to dissect the GvL effect from potentially lethal GvHD T-cell depletion will be essential (16). Studies on recipients of chemotherapy or autologous BMT for AML are underway along with allogeneic BMT studies in non-human primates (in collaboration with Wagemaker and Van Bekkum in Holland).

Conclusions and recommendations. Although it is now some 20 years since the first studies in rodents by Van Bekkum's group T-cell depletion in human allogeneic BMT is still in its infancy.

Some firm conclusions can be drawn. First, GvHD both acute and chronic is preventable in HLA matched and probably mismatched BMT, although further refinements in the technology of T-cell depletion are required. Our preliminary studies using HLA matched unrelated donors are equally encouraging. Second, rejection is largely preventable; encouraging results are being seen in our HLA matched unrelated donor transplant programme using pre-transplant conditioning with anti-T McAbs. Finally, it seems likely that the fears about the immune reconstitution of patients were unfounded and that within a short period the safe application of cytokines will allow us not only to accelerate the bone marrow recovery but also to boost the immune function.

Of more concern is the question of leukaemic relapse. T-cell depletion has exposed the inadequacy of conventional conditioning regimens used for immonusuppression and eradication of leukaemia and has both confirmed and extended the role of GvL. Although there is, no doubt, some scope for improvement in the anti-leukaemic conditioning, we feel that this might remain limited. Our observations, both in vivo and in vitro, suggest that an important role could be played by NK cells and we anticipate that their anti-leukaemic activity can be enhanced by the use of lymphokines such as IL2. We believe the evidence that GvL can be 'dissected' from GvHD by T lymphocyte depletion of the donor marrow is compelling and that it will only be with T-cell depletion that we can further, safely, enhance this effect.

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