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COMMENTARY

BONE MARROW THERAPY WITH MONOCLONAL ANTIBODIES

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Do monoclonal antibodies have a future in bone marrow therapy? If you judge by the numbers of papers appearing each month, there is at least a measure of enthusiasm, but a sober assessment of clinical results to date might leave you rather pessimistic. In fact, the very diversity of reagents and techniques that are now available makes critical evaluation difficult and might divert attention from more fundamental problems. Bone marrow therapy is a good testing ground for many of the new generation of biological drugs, but I will suggest that it is best used to evaluate reagents that might have wider applications.

At present bone marrow transplantation is used principally in the treatment of two categories of disease. The first consists of inherited or acquired defects in the haemopoietic system, for example, congenital immunodeficiencies, thalassaemia or aplastic anaemia. These diseases, and a few other rare metabolic disorders, can be cured by allogeneic marrow transplantation. It is usually necessary to treat the recipient with powerful immunosuppressive agents to prevent rejection of the foreign tissue. The other category is those disseminated malignant tumours (mainly leukaemias) which respond to intensive treatment by high dose chemotherapy and/or radiotherapy. Such treatment is toxic to bone marrow cells and would be lethal unless the patient were "rescued" by transfusion of fresh bone marrow. The "conditioning" used to destroy the tumour cells is also immunosuppressive and so prevents rejection. However, allogeneic marrow transplantation suffers from a serious problem, namely graft-versus-host disease. This is caused by mature T lymphocytes from the donor which are unavoidably harvested with the bone marrow. They recognise the recipient as foreign, attacking many organs including skin, liver and gut in a severe, often lethal, onslaught. Conventionally, immunosuppressive drugs such as steroids, methotrexate or cyclosporin A have been used to partly control this disease, but it has still afflicted up to 70% of patients even when the donors are siblings perfectly matched for major histocompatibility (MHC) antigens. When they are less well matched (and only 25–30% of patients have an MHC-matched sibling), then graft-versus-host disease is usually so severe that it prohibits transplantation.

Prevention of graft-versus-host disease

It has been known for some years from experiments in animals that graft-versus-host disease can be prevented by removal of mature T cells from the marrow [1–5]. One means of accomplishing this was to treat the cells with an antiserum which recognised T cells but spared the stem cells which repopulate the bone marrow. It was not easy to prepare these antisera so when monoclonal antibodies became available it was soon realised that those with appropriate specificity would be very useful since they would be available indefinitely. At first, rather little attention was given to the ways by which antibodies might destroy the unwanted cells. One of the first antibodies specific for T cells, OKT3 (mouse IgG2a), was merely mixed with the bone marrow in the hope that T cells would be opsonised for clearance by the recipient [6–8]. This was unsuccessful, posssibly because the antibody did not activate human effector mechanisms adequately, or because they were already too compromised by the conditioning regimen, or because the antibody was eliminated too quickly from the cell surface by antigenic modulation.

Attention turned to lysis of T cells *in vitro* using single antibodies or mixtures of different T cell specific antibodies together with complement. Unfortunately most antibodies were not lytic with human complement and so rabbit serum had to be used [9–12]. It is difficult to lyse cells with homologous complement because of the complement inhibitors that are present on cell membranes [13, 14]. These molecules are largely species-specific, and so heterologous complement is almost invariably more lytic [15, 16]. The use of rabbit serum introduced fresh problems because of batch-to-batch variability in potency and non-specific toxicity [17]. Therefore, we were pleased to discover an antibody (CAMPATH-1) which, though it was rather broadly reactive with both T and B lymphocytes, nevertheless gave very effective cell lysis with human complement [18]. Two factors in part responsible for this are the antibody isotype (rat IgM) and the rather high density of antigen (approx. 5×10^5 molecules/cell). CAMPATH-1 has now been employed by many transplant centres for T cell depletion (approx. 600 patients to date) because it is so simple to use [19–22]. There are, however, several alternative technologies for using monoclonal antibodies to deplete cells. These include coupling the antibodies to small magnetic particles, allowing cell removal by magnetism [23], or coupling the antibodies to plant or bacterial toxins which, once taken up by the cell, will inhibit protein synthesis and prevent replication [24–27]. A mixture of three anti-T cell immunotoxins has proven to be an effective tool for T cell depletion [28]. Although the initial preparation of the antibody–toxin conjugate may be slightly laborious, its one-step use is attractive.

Monoclonal antibodies do not provide the only route for depletion of T cells from bone marrow;

there are physical methods such as centrifugation [29, 30] as well as other immunological techniques such as lectin-agglutination and erythrocyte-rosetting [31–33]. In practice these alternatives are rather more cumbersome and would probably not be widely used once suitable monoclonal reagents were easily available.

From the patient's point of view the precise technology is relatively unimportant. Essentially similar results have been obtained with all of the effective means of T cell depletion [34]. The incidence and severity of graft-versus-host disease have been reduced substantially both in MHC-matched and in partly mismatched transplants. To illustrate this I may be forgiven for quoting only the figures from our own collaborative study of T cell depletion with CAMPATH-1 since this is the largest uniform series [22]. In 282 patients with leukaemia transplanted from MHC-matched siblings, the incidence of moderate to severe graft-versus-host disease (grade 2–4) was 12%. This compares with an incidence of about 45% with conventional immunosuppression [35]. The incidence and severity of chronic graft-versus-host disease were also much lower than before, and furthermore, this was accomplished even when maintainance immunosuppression was not given.

However, a new problem, graft failure, was observed which affected about 14% of the patients and was a significant cause of death. A comparable frequency of graft failure occurs with virtually all other protocols of T cell depletion [34], and several lines of evidence suggest that it is due to immune rejection of the donor marrow by residual host cells, probably T lymphocytes. Graft failure was more common in mismatched transplants (55%) but rare in autologous transplants (2%) when the same purging technique was used. Alloreactive recipient T cells can survive conditioning [36] and have been detected in several patients with graft failure [37, 38]. This problem was predicted from animal experiments which showed that engraftment is more difficult when marrow is depleted of T cells [39, 40]. It is generally supposed that there is a reciprocal relationship between host-versus-graft and graft-versus-host reactions with the intensity of both depending on the genetic disparity between donor and recipient. If this is true, we expect that extra immunosuppression of the recipients will overcome the problem of rejection. There have been some clinical trials in which the chemotherapy or radiotherapy has been escalated but both are already near the maximum tolerable limits and at best only modest improvements have been made [20, 41, 42]. The availability of monoclonal antibodies which recognise the cells responsible for rejection has prompted studies of their use *in vivo* as alternative immunosuppressants which might act synergistically with current conditioning regimens. We have explored this possibility by experiments in mice using rat antibodies which are known to be very effective at depleting their target cells *in vivo* [43]. These antibodies are specific for the mouse CD4 and CD8 molecules which are markers of the class II restricted (helper) and class I restricted (cytotoxic/suppressor) subsets of T cells. By depleting T cells from both donors and recipients with the combination of antibodies, it was possible to achieve full engraftment without rejection or graft-versus-host disease even when the donor and recipient had different MHC antigens. Depletion of only one subset of T cells from the donor did not prevent graft-versus-host disease, and likewise depletion of only one subset from the recipient did not prevent rejection. This is important because there have been suggestions that it might be possible to gain a more favourable result just by depletion of one or the other subset of T cells in the donor marrow.

Recently we developed a rat IgG2b antibody with a very similar specificity to the original CAMPATH-1 [44]. Rat IgG2b is the most potent IgG subclass for fixing human complement and binding to human Fc receptors, and the new antibody (CAMPATH-1G) has proven to be very effective at clearing lymphocytes *in vivo*. Trials are in progress to establish whether it will be valuable for preventing graft rejection.

At present the incidence of graft rejection virtually negates the advantage gained in elimination of graft-versus-host disease, but there is optimisim that, by use *in vivo* of the same or similar antibodies to those used for the marrow purging, it may be overcome. This will be of great benefit to patients with non-malignant disease who can expect to be cured if a satisfactory graft is established. In this context I should also mention the use of an antibody against the lymphocyte functional molecule LFA-1 (CD11a/CD18) present on most white blood cells. This has been used to prevent graft rejection in mismatched transplants for congenital immunodeficiencies [45]. The antibody blocks many immune activities *in vitro* but does not appear to deplete the target cells *in vivo*; its mode of action in promoting long-term acceptance of the foreign graft is presumably rather subtle since it was only administered for a short time around the transplant. There are clearly more ways of preventing an immune response than just by ablating the cells.

In leukemia patients another problem is potentially of more concern than even graft rejection. This is the possible increase in leukaemia relapse which might be seen following T cell depletion. It has long been thought that allogeneic bone marrow transplantation may contribute directly to leukaemia eradication because of the aggressive action of the donor immune system—the so-called graft-versus-leukemia effect. There is considerable experimental literature in support of this concept [46–48] though many animal studies use transplantable tumours which may not be ideal models of the clinical spectrum of disease. It seemed reasonable that much of the graft-versus-leukaemia effect was straightforward alloreactivity; if so, it should be most prominent in patients who suffered graft-versus-host disease, who would therefore have a lower risk of relapse. Two seminal papers from the Seattle team addressed this question and showed that there was indeed a correlation between relapse and lack of graft-versus-host disease in patients with acute leukaemia [49, 50]. This has been confirmed in a recent update [51]; furthermore, there was a significantly higher incidence of relapse after transplants from identical twins [52].

More recently, evidence that donor T cells play a

role in prevention of relapse comes from studies of patients with chronic granulocytic leukaemia (CGL). This is a tumour of the haemopoietic stem cell or something close to it and may differ substantially from other leukaemias which are usually committed to a particular lineage. Before T cell depletion the relapse rate following allogeneic transplantation for CGL was gratifyingly low and most patients were considered cured if they avoided graft-versus-host disease and other early complications [53, 54]. However, following T cell depletion some centres reported a substantially higher incidence of relapse [20, 55]. Documentation of relapse in CGL is unusual because it is possible for a patient to be apparently normal in all respects except that the malignant clone can be shown (by chromosome markers) to be active. In some patients this situation is transient, but in many it progresses to haematological relapse. Whether cytogenetic disease or haematological disease was considered, the same depressing result was seen. In the study by CAMPATH-1 users there were 100 patients with CGL transplanted in the first chronic phase [22]. Although a minority suffered graft-versus-host disease (15% grade 1, 17% grade 2–4), if the original graft-versus-leukaemia concept were true, we would still have expected these patients to be at lower risk of relapse. This, however, was not the case, but rather relapse correlated with slow engraftment (measured by the time post-transplant when the blood neutrophil count first rose above 0.5×10^9/L). This finding supported an alternative explanation for the graft-versus-leukaemia effect, which we have termed "haemopoietic competition" [56]. We assume that during the initial period of aplasia there is an unusually high rate of stem cell replication to fill the empty bone marrow. Replication of both donor and residual recipient stem cells will occur and superimposed on this process is the complex interaction of donor and recipient T cells. In the past the donor would have had the overwhelming advantage because recipient stem cells and T cells are largely ablated by the pre-transplant conditioning. However, if the donor marrow is depleted of T cells, the few residual recipient T cells may be able to mount a rejection response. In extreme cases this will result in complete graft rejection; in others it may only slow the rate of donor engraftment, giving a greater opportunity for recipient (malignant) stem cells to expand and thus be more likely to cause relapse when they eventually start to differentiate. On this model we would expect to find cases of mixed chimerism more frequently after T cell depletion, and preliminary reports suggest that this is so [57, 58].

Of course we do not imagine that only one or the other explanation of the graft-versus-leukaemia effect will prove to be true. It is likely that both mechanisms are relevant to some extent, the balance possibly being different in each disease. However, it is important to know which mechanism predominates. If it is the direct attack of donor T cells on the leukaemic cells, then the strategy of T cell depletion will need to be re-thought. If, on the other hand, haemopoietic competition is more important, then we predict that extra immunosuppression of the recipient should speed engraftment and increase the proportion of "good" cells in the stem cell pool, thus reducing the likelihood of relapse.

So far I have neglected the possible contribution of non-T immune cells, e.g. NK cells. There have been suggestions that these cells exert a direct anti-leukaemic effect which could be spared by some depletion regimens that are targeted specifically at T cells. The case is still to be proved clinically, and so far there have been no studies on the numbers of such cells transplanted after different purging techniques. My own view is that the bulk of donor anti-leukaemic activity will be indistinguishable from plain alloreactivity, at least until we have appropriate ways of manipulating cells according to their specific antigen receptors.

Autologous marrow transplantation

The problems of graft-versus-host disease and rejection have so far proven to be such severe obstacles to allogeneic marrow transplantation that it is effectively limited to the minority of patients with MHC-matched sibling donors. Recently, progress has been made in identifying unrelated matched volunteers but, however large a donor panel were available, this would not always be successful. An alternative way of rescuing patients from the marrow toxicity of intensive anti-tumour regimens is the reinfusion of marrow taken out before the treatment was started [59–61]. The problem is that there is a risk of reinfusing viable tumour cells. Purging the marrow of tumour cells has been a fertile area of research in recent years [62].

When monoclonal antibodies first became available, there was some optimism that tumour-specific reagents could be found but, with few exceptions, the hope was short-lived. However, there is a panoply of antibodies that recognise antigens specific to cell lineages of particular stages of differentiation and many of these may be useful for separating tumour cells from stem cells irrespective of whether normal, differentiated cells are also recognised. Antibodies to leucocytes have their own triennial workshop where reagents are exchanged and a systematic nomenclature is agreed upon [63]. Similar workshops are in operation for other cell types, e.g. small lung cell carcinoma—a tumour of interest for autologous marrow transplantation [64]. Cell surface antigens are always heterogeneously expressed and so even in a clonal population of cells there will be some which express less antigen than others. If you also consider that individual tumours often differ in their exact phenotype, it is clear that a mixture of antibodies with different specificities will be needed if all the tumour cells are to be targeted.

The mechanism of cell depletion also needs to be considered. By analogy with depletion of T cells from bone marrow, it might appear that complement lysis would be adequate and, indeed, several different mixtures of antibodies have been proposed for purging lymphoblastic leukaemia cells with rabbit or human complement [65–67]. Often, however, this method has not been favoured, mainly because few antigens are good targets for complement-mediated lysis [68]. Also, some tumour cells appear to have extra resistance to lysis [69]. Therefore, there have

been great efforts to optimise alternative purging methods. One is the attachment of antibodies to magnetic particles (possibly indirectly, by means of an antiglobulin) so that the coated cells can then be separated with a powerful magnet. This technique was pioneered by Kemshead and coworkers who used a mixture of antibodies to remove neuroblastoma cells from marrow [70]. The method has also been applied to leukaemia [71, 72].

Another purging method is to use immunotoxins. Ricin has been the favoured cell poison, and a considerable amount of work has been done to characterise various antibody-ricin conjugates [24, 27]. Once inside the cell, ricin and other ribosome-inactivating proteins are extraordinarily potent, but one of the main problems has been that different antibody conjugates differ considerably in their abilities to promote uptake of the toxin into the cytoplasm. A popular antibody conjugate is T101(CD5)-ricin which has been used to eliminate malignant T cells [73].

The technological stumbling block of marrow purging is how to assess the efficacy *in vitro* and so to decide which of several alternative techniques is the best. The clinical difficulty is how to tell whether it is of any real value—since it is virtually impossible to know whether a later relapse might be due to inadequate purging or to residual disease in the patient after conditioning.

The first problem has been tackled in two ways. First, normal bone marrow can be contaminated deliberately with a tumour cell line which can be assayed very sensitively by limiting dilution cloning [62]. The degree of depletion of this line is used as a measure of the efficacy of the technique. The snag is that the chosen technique may not work so well with fresh tumours. Alternatively, sensitive staining techniques are sought which can reveal residual tumour cells before and after the antibody treatment [67, 74]. At best such assays can detect about 0.1% tumour cells—which could still represent up to 10^7 cells reinfused. In practice, this amount could be small in comparison with residual disease in the patient.

In my view the more serious problem is how to demonstrate whether purging is really needed at the current stage in the art of pre-transplant conditioning. It is an unfortunate fact that those tumours which have been most extensively treated by autologous transplantation (e.g. acute lymphoblastic leukaemia) exhibit a substantial incidence of relapse after allogeneic transplantation. This proves that current conditioning regimens are not adequate to eliminate residual disease even with the possible bonus of the graft-versus-leukaemia effect. Many of the other diseases that are being treated by autologous transplantation with marrow purging (e.g. lymphoma, small cell lung carcinoma, neuroblastoma) have not often been treated by allogeneic transplantation so we do not have a comparable baseline, but there is no reason to suppose that the same difficulty will not apply. If relapse due to inadequate conditioning did not occur, it would be a simple matter to prove the need (or otherwise) of marrow purging. In practice, very substantial randomised trials will be needed to show a significant margin of improvement from purging compared with no purging, and those studies reported so far have shown no difference [75]. Faced with the probability that purging does no harm, save in cost and effort, it is unlikely that such trials will be completed.

There are some situations where the cause of relapse might be determined. Tumour cells that have been subject to irradiation will often exhibit extra chromosomal abnormalities at relapse which might distinguish them from cells reintroduced with the marrow [76]. Also, in solid tumours it might be expected that relapse at the original sites would indicate inadequate conditioning whereas relapse at different sites could be due to reinfused tumour cells. At least one group has reported a high frequency of relapse at original tumour sites following autologous transplantation for Hodgkin's lymphoma [77], suggesting again that purging is not currently the main problem.

If inadequate pre-transplant conditioning is the main cause of treatment failure, surely more attention should be given to devising new anti-tumour reagents that can be used *in vivo*. Monoclonal antibodies are, of course, potential candidates, and when antibodies against cell surface differentiation antigens first became available, there were several attempts at tumour therapy with them [78]. There are two main strategies for using monoclonal antibodies: either they can be administered alone, relying on physiological effector mechanisms (complement lysis or antibody-dependent cell-mediated cytotoxicity) or they can be coupled to some cytotoxic substance (e.g. plant or bacterial toxin or radioisotope). There is a long history of disappointing results with polyclonal antisera in serotherapy [79], and initial results with unconjugated monoclonal antibodies have, on the whole, been no better [78]. Therefore, most attention is now being given to toxin-antibody conjugates [24, 27] or radiolabelled antibodies [80]. Nevertheless, the first clinical results have still not been particularly impressive, and there is clearly a long way to go before Ehrlich's "magic bullet" is obtained. Immunotoxins are not without potentially dangerous side-effects, and there are theoretical limitations to the scope of therapy with radiolabelled antibodies because of the nonspecific effects of the radiation [81]. My view is that physiological effector mechanisms may still play an important role, and I am encouraged in that by animal experiments which show that unconjugated antibodies can achieve substantial cell depletion [82]. Recently we tested the antibody CAMPATH-1G which seems to combine an optimal specificity for an abundant lymphocyte antigen with an optimal Fc (rat IgG2b). Unlike many others, this antibody gave substantial clearance of leukaemic lymphocytes *in vivo*, and it may be useful for serotherapy of this type of tumour as well as for immunosuppression. It could hardly have been developed without the extensive studies of T cell depletion from bone marrow with the equivalent IgM. They had convincingly demonstrated its reactivity with lymphocytes and, importantly, its lack of reactivity with stem cells. I would therefore argue the value of purging protocols which potentially have wider applications. This means using antibody specificities and purging techniques that could be also

used *in vivo*. Immunotoxins might qualify by this criterion, but immunomagnetic depletion would be a non-starter.

Future directions

If the addition of monoclonal antibodies or other biologic effectors to conditioning regimens will give better eradication of disseminated tumours, then autologous marrow transplantation with appropriate purging of tumour cells will become a very useful therapy. To avoid the need for many different antibodies directed against different tumour types, some form of positive selection of stem cells would be logical. This might be achieved using antibodies specific for only immature haemopoietic cells, for example My10/ICH3 [83]. A shorter-term goal is to overcome the twin problems of rejection and graft-versus-host disease in allogeneic transplantation. If this could be reliably achieved with unrelated or only partly matched family donors, then the range of diseases that could be treated by marrow transplantation would be widened significantly. First, it would be possible to offer a cure for congenital diseases such as thalassaemia where at present the risks of transplantation are usually considered too great. Second, marrow transplantation might also be used as a way of creating donor-specific tolerance prior to an organ transplant. This would mean that long-term immunosuppression would not be needed. There are good experimental precedents for this concept [43, 84, 85], but so far it has not proven to be clinically practicable because of the problems of rejection and GVHD. Finally, we could imagine that ablation of the immune system followed by allogeneic marrow transplantation could be used to treat chronic autoimmune diseases, supposing that the new immune system could develop in a tolerogenic rather than a stimulatory environment. When some of these objectives have been achieved, then bone marrow therapy will really have come of age.

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REFERENCES

1. Dicke KA, van Hooft J and van Bekkum DW, The selective elimination of immunologically competent cells from bone marrow and lymphatic cell mixtures. *Transplantation* **6**: 562–570, 1968.
2. Rodt H, Thierfelder S and Eulitz M, Anti-lymphocyte antibodies and marrow transplantation. III Effect of heterologous anti-brain antibodies on acute secondary disease in mice. *Eur J Immunol* **4**: 25–29, 1974.
3. Reisner Y, Itzicovitch L, Meshorer A and Sharon N, Haemopoetic stem cell transplantation using mouse bone marrow and spleen cells fractionated by lectins. *Proc Natl Acad Sci USA* **75**: 2933–2936, 1978.
4. Vallera DA, Soderling CCR, Carlson GJ and Kersey JH, Bone marrow transplantation across major histocompatibility barriers in mice. Effect of elimination of T cells from donor grafts by pretreatment with monoclonal anti-Thy-1 plus complement or antibody alone. *Transplantation* **31**: 218–222, 1981.
5. Wagemaker G, Heidt PJ, Merchav S and van Bekkum DW, Abrogation of histocompatibility barriers to bone marrow transplantation in rhesus monkey. *Exp Hematol* **9**, (Suppl. 9): 95, 1981.
6. Filipovitch AH, Ramsay NKC, Warkentin PI, McGlave PB, Goldstein G and Kersey JH, Pretreatment of donor bone marrow with monoclonal antibody OKT3 for prevention of acute graft versus host disease in allogeneic histocompatible bone marrow transplantation. *Lancet* **1**: 1266–1269, 1982.
7. Prentice HG, Blacklock HA, Janossy G, Bradstock KF, Skeggs D, Goldstein G and Hoffbrand AV, Use of anti-T cell monoclonal antibody OKT3 to prevent acute graft versus host disease in allogeneic bone marrow transplantation for acute leukemia. *Lancet* **1**: 700–703, 1982.
8. Hayward AR, Murphy S, Githens J, Troup G and Ambruso D, Failure of a pan-reactive anti-T cell antibody OKT 3, to prevent graft versus host disease in severe combined immunodeficiency. *J Pediatr* **100**: 665, 1982.
9. Trigg ME, Billing R, Sondel PM, Dikerman JD, Erickson C, Finlay JL, Bozdech M, Hong R, Padillanash H and Terasaki P, Depletion of T cells from human bone marrow with monoclonal antibody CT-2 and complement. *J Biol Response Mod* **3**: 406–412, 1984.
10. Prentice HG, Blacklock HA, Janossy G, Gilmore MJML, Price-Jones L, Tidman N, Trejdosiewicz LK, Skeggs DBL, Panjwni D, Ball S, Graphakos S, Patterson J and Hoffbrand AV, Depletion of T lymphocytes in donor marrow prevents significant graft versus host disease in matched allogeneic leukemic marrow transplant recipients. *Lancet* **1**: 472–476, 1984.
11. Herve P, Flesch M, Cahn JY, Racadot E, Plouvier E, Lamy B, Rozenbaum A, Noir A, des Floris RL and Peters A, Removal of marrow T cells with OKT3–OKT11 monoclonal antibodies and complement to prevent acute graft versus host disease. *Transplantation* **39**: 138–143, 1985.
12. Martin PJ, Hansen JA, Storb R and Thomas ED, A clinical trial of *in vitro* depletion of T cells in donor marrow for prevention of acute graft-versus-host disease. *Transplant Proc* **17**: 486–487, 1985.
13. Medof ME, Kinoshita R and Nuzzenzweig V, Inhibition of complement activation on the surface of cells after incorporation of decay-accelerating factor (DAF) into their membranes. *J Exp Med* **160**: 1558–1578, 1984.
14. Shin ML, Hansch G, Hu VW and Nicholson-Weller A, Membrane factors responsible for homologous species restriction of complement-mediated lysis: Evidence for a factor other than DAF operating at the stage of C8 and C9. *J Immunol* **136**: 1777–1782, 1986.
15. Hansch GM, Hammer CH, Vanguri P and Shin ML, Homologous species restriction in lysis of erythrocytes by terminal complement proteins. *Proc Natl Acad Sci USA* **78**: 5118–5121, 1981.
16. Houle JJ and Hoffmann EM, Evidence for the restriction of the ability of complement to lyse homologous erythrocytes. *J Immunol* **133**: 1444–1452, 1984.
17. Richiardi P, Amoroso A, Crepaldi T and Curtoni ES, Cytolytic activity of monoclonal antibodies strongly depends on rabbit complement used. *Tissue Antigens* **17**: 368–371, 1981.
18. Hale G, Bright S, Chumbley G, Hoang T, Metcalf D, Munro AJ and Waldmann H, Removal of T cells from bone marrow for transplantation: A monoclonal anti-lymphocyte antibody that fixes human complement. *Blood* **62**: 873–882, 1983.
19. Waldmann H, Or R, Hale G, Weiss L, Cividalli G, Samuel S, Manor D, Brautbar C, Polliack A, Rachmilewitz EA and Slavin S, Elimination of graft versus host disease by *in vitro* depletion of alloreactive lymphocytes using a monoclonal rat anti-human lymphocyte antibody (CAMPATH-1). *Lancet* **2**: 483–486, 1984.
20. Apperley JF, Jones L, Hale G, Waldmann H, Hows J,

Rombos Y, Tsataalas C, Marcus RE, Goolden AWG, Gordon-Smith EC, Catovsky D, Galton DAG and Goldman JM, Bone marrow transplantation for patients with chronic myeloid leukaemia: T cell depletion with Campath-1 reduces the incidence of graft-versus-host disease but may increase the risk of leukaemic relapse. *Bone Marrow Transplant* **1**: 53–66, 1986.

21. Heit W, Bunjes D, Weisneth M, Schmeiser T, Arnold R, Hale G, Waldmann H and Heimpel H, *Ex vivo* T-cell depletion with the monoclonal antibody Campath-1 plus human complement effectively prevents acute graft-versus-host disease in allogeneic bone marrow transplantation. *Br J Haematol* **64**: 479–486, 1986.
22. Hale G, Cobbold S and Waldmann H, for CAMPATH-1 users. T cell depletion with CAMPATH-1 in allogeneic bone marrow transplantation. *Transplantation* **45**: 753–759, 1988.
23. Gee AP, Lee C, Sleasman JW, Madden M, Ugelstad J and Barrett DJ, T lymphocyte depletion of human peripheral blood and bone marrow using monoclonal antibodies and magentic microspheres. *Bone Marrow Transplant* **2**: 155–164, 1987.
24. Thorpe PE, Edwards DC, Davies AJS and Ross WCJ, Monoclonal antibody-toxin conjugates: Aiming the magic bullet. In: *Monoclonal Antibodies in Clinical Medicine* (Eds. Fabre J and McMichael A), pp. 167–201. Academic Press, London, 1982.
25. Poynton CH and Reading CL, Monoclonal antibodies: The possibilities for cancer therapy. *Exp Biol* **43**: 13–33, 1984.
26. Blair AH and Ghose TI, Linkage of cytotoxic agents to immunoglobulins. *J Immunol Methods* **59**: 129–143, 1983.
27. Vitetta ES and Uhr JW, The potential use of immunotoxins in transplantation, cancer therapy, and immunoregulation. *Transplantation* **37**: 535–538, 1984.
28. Filipovitch AH, Vallera DA, Youle RJ, Haake R, Blazar BR, Arthur D, Neville DM, Ramsay NK, McGlave P and Kersey JH, Graft-versus-host disease prevention in allogeneic bone marrow transplantation from histocompatible siblings. A pilot study using immunotoxins for T cell depletion of donor bone marrow. *Transplantation* **44**: 62–69, 1987.
29. Lowenberg B, Wagemaker G, van Bekkum DW, Sizoo W, Sintnicolaas K, Hendriks WDH and Hagenbeek A, The use of gradient separated stem cell grafts in HLA matched donor-recipient bone marrow transplantation. *Exp Hematol* **13** (Suppl. 17): 119, 1985.
30. Noga SJ, Donnenberg AP, Schwartz CL, Strauss LC, Civin CI and Santos GW, Development of a simplified counterflow centrifugation elutriation procedure for depletion of lymphocytes from human bone marrow. *Transplantation* **41**: 214–219, 1986.
31. Reisner Y, Kapoor N, Kirkpatrick D, Pollack MS, Dupont B, Good RA and O'Reilly RJ, Transplantation for acute leukaemia with HLA-A and B nonidentical parental marrow cells fractionated with soybean agglutinin and sheep red blood cells. *Lancet* **2**: 327–331, 1981.
32. Levinsky RJ, Davies EG, Butler M, Linch DC and Goldstone AH, Problems of mismatched bone marrow transplantation for severe combined immunodeficiency using soybean lectin fractionation. *Exp Hematol* **11** (Suppl. 13): 37, 1983.
33. Friedrich W, Goldmann SF, Blutters-Sawatzki R, Ebell W, Raghavachar A, Kleihuer E and Kubanek B, T cell depletion of marrow for the prevention of graft versus host disease. *Exp Hematol* **13** (Suppl. 17): 121–122, 1985.
34. O'Reilly RJ, Current developments in marrow transplantation. *Transplant Proc* **19**: 92–102, 1987.
35. Bortin MM, Acute graft-versus-host disease following bone marrow transplantation in humans: Prognostic factors. *Transplant Proc* **19**: 2655–2657, 1987.
36. Butturini A, Seeger RC and Gale RP, Recipient immune competent T lymphocyte can survive intensive conditioning for bone marrow transplantation. *Blood* **68**: 954–956, 1986.
37. Bunjes D, Wiesneth M, Schmeiser Th, Arnold R and Heit W, An immunological analysis of three cases of bone marrow failure associated with Campath-1-incubation. *Exp Hematol 13* (Suppl. 17): 118, 1985.
38. Kernan NA, Flomenberg N, Dupont B and O'Reilly RJ, Graft rejection in recipients of T-cell-depleted HLA-identical marrow transplants for leukemia. Identification of host-derived antidonor allocytotoxic T lymphocytes. *Transplantation* **43**: 842–847, 1987.
39. Vriesendorp HM, Klapwijk WM, Heidt DJ, Hogeweg B, Zurcher C and van Bekkum DW, Factors controlling the engraftment of transplanted dog bone marrow cells. *Tissue Antigens* **20**: 63, 1982.
40. Wagemaker G, Vriesendorp HM and van Bekkum DW, Successful bone marrow transplantation across major histocompatibility barriers in rhesus monkeys. *Transplant Proc* 13: 875–880, 1981.
41. Trigg ME, Billing R, Sondel PM, Exten R, Hong R, Bozdech MJ, Horowitz SD, Finlay JL, Moen R, Longo W, Erickson C and Peterson A, Clinical trial depleting T lymphocytes from donor marrow for matched and mismatched allogeneic bone marrow transplants. *Cancer Treat Rep* **69**: 377–386, 1985.
42. Martin PJ, Hansen JA, Buckner CD, Sanders JE, Deeg HJ, Stewart P, Appelbaum FR, Clift R, Fefer A, Witherspoon RP, Kennedy MS, Sullivan KM, Flournoy, N, Storb R and Thomas ED, Effects of *in vitro* depletion of T cells in HLA-identical allogeneic marrow grafts. *Blood* **66**: 664–672, 1985.
43. Cobbold S, Martin G and Waldmann H, Monoclonal antibodies for the prevention of graft-versus-host disease and marrow graft rejection: The depletion of T-cell subsets *in vitro* and *in vivo*. *Transplantation* **42**: 239–247, 1986.
44. Hale G, Cobbold SP, Waldmann H, Easter G, Matejtschuk P and Coombs RRA, Isolation of low-frequency class-switch variants from rat hybrid myelomas. *J Immunol Methods* **103**: 59–67, 1987.
45. Fischer A, Griscelli C, Blanche S, Le-Deist F, Veber F, Lopez M, Delaage M, Olive D, Mawas C and Janossy G, Prevention of graft failure by an anti-HLFA-1 monoclonal antibody in HLA-mismatched bone marrow transplantation. *Lancet* **2**: 1058–1061, 1986.
46. O'Kunewick JP and Meredith RF (Eds.), *Graft-versus-leukemia in Man and Animal Models*. CRC Press, Boca Raton, FL, 1981.
47. Weiss L, Morecki S, Vitetta ES and Slavin S, Suppression and elimination of BCL1 leukemia by allogeneic bone marrow transplantation. *J Immunol* **130**: 2452–2455, 1983.
48. Truitt RL, Shih CC and LeFever AV, Manipulation of graft-versus-host disease for a graft-versus-leukemia effect after allogeneic bone marrow transplantation in AKR mice with spontaneous leukemia/lymphoma. *Transplantation* **41**: 301–310, 1986.
49. Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD and Storb R, Antileukemic effect of graft-versus-host disease in human recipients of allogeneic marrow grafts. *N Engl J Med* **300**: 1068–1073, 1979.
50. Weiden PL, Sullivan KM, Fluornoy N, Storb R, Thomas ED and the Seattle Marrow Transplant Team, Antileukemic effect of chronic graft-versus-host disease: Contribution to improved survival after allogeneic marrow transplantation. *N Engl J Med* **304**: 1529–1533, 1981.

51. Sullivan KM, Fefer A, Witherspoon R, Storb R, Buckner CD, Weiden P, Schoch G and Thomas ED, Graft-versus-leukemia in man: Relationship of acute and chronic graft-versus-host disease to relapse of acute leukemia following allogeneic bone marrow transplantation. *Prog Clin Biol Res* **244**: 391–399, 1987.
52. Fefer A, Sullivan KM, Weiden P, Buckner CD, Schoch G, Storb R and Thomas ED, Graft versus leukemia effect in man: The relapse rate of acute leukemia is lower after allogeneic than after syngeneic marrow transplantation. *Prog Clin Biol Res* **244**: 401–408, 1987.
53. Speck B, Bortin MM, Champlin R, Goldman JM, Herzig RH, McGlave PB, Messner HA, Weiner RS and Rimm AA, Allogeneic bone marrow transplantation for chronic myelogenous leukaemia. *Lancet* **1**: 665–668, 1984.
54. Thomas ED, Clift RA, Fefer A, Appelbaum FR, Beatty P, Bensinger WI, Buckner CD, Cheever MA, Deeg J, Doney C, Flournoy N, Greenberg P, Hansen JA, Martin P, McGuffin R, Ramberg R, Sanders JE, Singer J, Stewart P, Storb R, Sullivan K, Weiden PL and Witherspoon R, Marrow transplantation for the treatment of chronic myelogenous leukaemia. *Ann Intern Med* **104**: 155–163, 1986.
55. Papa G, Arcese W, Mauro FR, Bianchi A, Alimena G, De Felice L, Isacchi D, Pasqualetti D, Malagnino F, Purpura M, Girelli G and Mandelli F, Standard conditioning regimen and T-depleted donor bone marrow for transplantation in chronic myeloid leukamia. *Bone Marrow Transplant* **1** (Suppl. 1): 97, 1986.
56. Waldman H, Hale G and Cobbold S, The immunobiology of bone marrow transplantation. In: *Leucocyte Typing III: White Cell Differentiation Antigens* (Ed. McMichael AJ), pp. 932–937. Oxford University Press, Oxford, 1987.
57. Janssen J, Drenthe-Schonk A, van Dijk B, Bloo J, Kunst V and de Witte T, Erythrocyte repopulation after allogeneic bone marrow transplantation using lymphocyte depleted marrow from histocompatible siblings. *Bone Marrow Transplant* **1** (Suppl. 1): 239–240, 1986.
58. Mascret B, Bertheas MF, Lafage M, Blaise D, Maranichi D, Fraisse J and Carcassone Y, Cytogenetic evidence of partial chimerism after T cell depleted allogeneic transplantation in leukaemic matched patients. *Bone Marrow Transplant* **1** (Suppl. 1): 235–236, 1986.
59. Dicke KA and Spitzer G, Clinical studies of autografting in acute lymphocytic leukaemia. *Clin Haematol* **15**, 85–103, 1986.
60. Philip T, Pinkerton R, Hartmann O, Patte C, Philip I, Biron P and Favrot M, The role of massive therapy with autologous bone marrow transplantation in Burkitt's lymphoma. *Clin Haematol* **15**: 205–217, 1986.
61. Pinkerton R, Philip T, Bouffet E, Lashford L and Kemshead J, Autologous bone marrow transplantation in paediatric solid tumours. *Clin Haematol* **15**: 187–203, 1986.
62. Jansen J, Falkenburg JHF, Stepan DE and LeBien TW, Removal of neoplastic cells from autologous bone marrow grafts with monoclonal antibodies. *Semin Hematol* **21**: 164–181, 1984.
63. McMichael AJ (Ed.), *Leucocyte Typing III: White Cell Differentiation Antigens*. Oxford University Press, Oxford, 1987.
64. Souhami RL, Beverley PC and Bobrow LG, Antigens of small-cell lung cancer. First international workshop. *Lancet* **2**: 325–326, 1987.
65. Nakamura H, Hirayama F, Tatsumi Y, Tani Y, Kubota Y, Kanakura Y, Oguma S, Ueda T, Shibata H and Masaoka T, *In vitro* treatment of human acute lymphocytic leukemia cells in bone marrow with a cocktail of monoclonal antibodies. *Jpn J Clin Oncol* **14** (Suppl. 1): 537–542, 1984.
66. Sugita K, Majdic O, Stockinger H, Holter W, Koller U, Peschel C and Knapp W, Use of cocktail of monoclonal antibodies and human complement in selective killing of acute lymphocytic leukaemia cells. *Int J Cancer* **37**: 351–357, 1986.
67. Janossy G, Campana D, Galton J, Grob J-P, Burnett A, Hann I, Simonsson B and Totterman T, Applications of monoclonal antibodies in bone marrow transplantation. In: *Leucocyte Typing III: White Cell Differentiation Antigens* (Ed. McMichael AJ), pp. 942–948. Oxford University Press, Oxford, 1987.
68. Hale G and Waldmann H, Human complement fixing antibodies in transplantation. In: *Hybridoma Technology in the Biosciences and Medicine* (Ed. Springer TA), pp. 453–471. Plenum Press, New York, 1985.
69. Ohanian SH, Borsos T and Rapp HJ, Lysis of tumour cells by antibody and complement 1. Lack of correlation between antigen content and lytic susceptibility. *J Natl Cancer Inst* **50**: 1313–1320, 1973.
70. Treleavan JG, Gibson FM, Ugelstad J, Rembaum A, Philip T, Caine GD and Kemshead JT, Removal of neuroblastoma cells from bone marrow with monoclonal antibodies conjugated to magnetic microspheres. *Lancet* **1**: 70–73, 1984.
71. Poynton CH, Dicke KA, Culbert S, Frankel LS, Jagannath S and Reading CL, Immunomagnetic removal of CALLA-positive cells from human bone marrow. *Lancet* **1**: 524, 1983.
72. Kemshead JT, Treleaven J, Heath L, Meara AO, Gee A and Ugelstad J, Monoclonal antibodies and magnetic microspheres for the depletion of leukaemia cells from bone marrow harvested for transplantation. *Bone Marrow Transplant* **2**: 133–140, 1987.
73. Gorin NC, Douay L, Laporte JP, Lopez M, Zittoun R, Rio B, David R, Stachowiak J, Jansen J, Cazellas P, Poncelet P, Liance MC, Voisin GA, Salmon D, LeBlanc G, Deloux J, Najman A and Duhamel G, Autologous bone marrow transplantation with marrow decontaminated by immunotoxin T101 in the treatment of leukemia and lymphoma: First clinical observations. *Cancer Treat Rep* **69**: 953–959, 1985.
74. Kemshead JT, Heath L, Gibson FM, Katz F, Richmond F, Treleavan J and Ugelstad J, Magnetic microspheres and monoclonal antibodies for the depletion of neuroblastoma cells from bone marrow: Experiences, improvements and observations. *Br J Cancer* **54**: 771–778, 1986.
75. Gorin NC, Herve P, Aegerter P, Goldstone A, Linch D, Maranichi D, Burnett A, Helbig W, Meloni G, Verdonck LF, Rizzoli V, Parlier Y, Auvert B and Goldman J, Autologous bone marrow transplantation for acute leukaemia in remission. *Br J Haematol* **64**: 385–395, 1986.
76. Baker MC, Lawler SD, Harris H, Barrett A and Powles RL, Radiation damage in patients treated by total body irradiation, bone marrow grafting and cyclosporin. *Radiat Res* **105**: 413–424, 1986.
77. Phillips GL and Reece DE, Clinical studies of autologous bone marrow transplantation in Hodgkin's disease. *Clin Haematol* **15**: 151–166, 1986.
78. Ritz J and Schlossman SF, Utilization of monoclonal antibodies in the treatment of leukemia and lymphoma. *Blood* **59**: 1–11, 1982.
79. Currie GA, Eighty years of immunotherapy: A review of immunologic methods used for the treatment of human cancer. *Br J Cancer* **26**: 141–153, 1972.
80. Badger CC and Bernstein ID, Prospects for monoclonal antibody therapy of leukemia and lymphoma. *Cancer* **58**: 587–589, 1986.
81. Vaughan AT, Anderson P, Dykes PW, Chapman CE and Bradwell AR, Limitations to the killing of tumors

using radiolabelled antibodies. *Br J Radiol* **60**: 567–572, 1987.
82. Qin S, Cobbold S, Tighe H, Benjamin R and Waldmann H, CD4 monoclonal antibody pairs for immunosuppression and tolerance induction. *Eur J Immunol* **17**: 1159–1165, 1987.
83. Watt SM, Karhi K, Gatter K, Furley AJ, Katz FE, Healy LE, Altass LJ, Bradley NJ, Sutherland DR, Levinsky R and Greaves MF, Distribution and epitope analysis of the cell membrane glycoprotein (HPCA-1) associated with human hemopoietic progenitor cells. *Leukemia* **1**: 417–426, 1987.
84. De Fazio SR, Hertner WC, Monaco AP and Gozzo JJ, Mouse skin graft prolongation with donor-strain bone marrow and antilymphocyte serum. *Transplantation* **41**: 26–28, 1986.
85. Thomas JM, Carver FM, Foil MB, Hall WR, Adams C, Fahrenbruch GB and Thomas FT, Renal allograft tolerance induced with ATG and donor bone marrow in outbred rhesus monkeys. *Transplantation* **36**: 104–105, 1983.