replacement for at least a year, and even then conventional adult hormone replacement should not be contemplated for at least another year. To move more quickly than this causes development of the breasts which is almost entirely confined to the nipple and areola—which causes great unhappiness. Whether in such patients history can be rerun with advantage is far from clear.

The problem with the medical care of patients in their teens is that in the hospital service such patients fall between the spheres of knowledge of physicians, including endocrinologists and oncologists, of gynaecologists, and of paediatricians. Family doctors may be more directly concerned, but unfortunately the education which most of us had on this difficult topic was woefully inadequate. Puberty is of such

great importance to patients that abnormalities of it deserve early recognition and skilled, if not complex, management.

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- 1 Marshall WA. Normal puberty. In: Brook CGD, ed. *Clinical paediatric endocrinology*. Oxford: Blackwell Scientific Publications, 1981:193-206.
- 2 Burns EC, Tanner JM, Preece MA, Cameron N. Final height and pubertal development in 55 children with idiopathic growth hormone deficiency treated for between 2 and 15 years with human growth hormone. *Eur J Pediatr* 1981;137:155-64.
- 3 Boyar RM, Finkelstein R, Roffwarg H, Kapen S, Weitzman E, Hellman L. Synchronisation of augmented luteinising hormone secretion with sleep during puberty. *N Engl J Med* 1972;287:582-6.
- 4 Stanhope RS, Adams J, Jacobs HS, Brook CGD. The induction of puberty by low dose pulsatile GnRH. *Pediatr Res* 1984;18:1210.

Regular Review

Graft versus host diseases: new versions of old problems?

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Bone marrow transplantation is now an accepted part of medical treatment. For example, chemotherapy achieves remissions in few adults with acute non-lymphocytic leukaemia and in virtually none in relapse. In contrast, bone marrow grafts from HLA compatible sibling donors have substantially improved the outlook for such patients.¹ The grafted cells take on the function of the recipient's own bone marrow which has been destroyed by total body irradiation and high dose cyclophosphamide. Bone marrow grafts are also being used increasingly to replace the non-functioning bone marrow of patients with aplastic anaemia. In addition, marrow transplantation is the treatment of choice in severe combined immunodeficiency and is being tried in other forms of inherited defects of bone marrow function.²

Siblings who are HLA identical are still the preferred donors, but unrelated donors are being selected more often, usually with the proviso that at least they have one HLA haplotype in common with the recipient and possess identical HLA-D determinants. These grafts from imperfectly matched donors require more immunosuppression to be successful—and are more likely to induce graft versus host disease.

Graft versus host disease has long been recognised as a major obstacle to successful transplantation. Its acute form, occurring within 100 days of transplantation and manifested by rash, hepatitis, diarrhoea, and delayed restoration of haemopoiesis and lymphopoiesis, was observed in 30-70% of patients in early transplantation programmes. Several factors have reduced its incidence, some concerned with the selection of donors and others with the management of the procedure itself and the aftercare of patients. With growing experience in ensuring the survival of grafts and in overcoming acute graft versus host disease the chronic disease has become increasingly common, with an incidence varying between 15% and 40% in different centres.

The clinical and pathological features of graft versus host disease are of interest not only for their practical importance to transplantation practice but as models of immunopathological processes which may operate in several spontaneous diseases of unknown aetiology. Indeed, this possibility was foreseen in pioneering studies in animal models of graft versus host disease in the 1960s.³

Chronic graft versus host disease appears between 70 and 400 days after transplantation and has well defined clinical features. Skin lesions are almost invariably present. At first these are inflammatory but later they take the form of subcutaneous fibrosis with telangiectasia and joint contractures; oral lesions resembling lichen planus, oesophageal strictures, and the sicca syndrome are common, while polymyositis and serositis are occasionally encountered.^{4,5} Severe obstructive airways disease has also been reported.⁶ There are definite similarities with diseases such as scleroderma—but there are also differences and, in particular, a virtual absence of renal abnormalities of the kind characteristic of scleroderma and systemic lupus erythematosus.⁷

Detailed histological analysis has confirmed the clinical distinction between the acute and chronic forms of graft versus host disease.⁸ Many features of the lesions in the acute form suggest that the target cells may be damaged by the products of donor lymphocytes and by natural killer cells and not simply by donor T lymphocytes. In contrast, chronic graft versus host disease is marked by florid cellular infiltration and vascular reactions resembling the lesions of primary connective tissue diseases.

Pathogenesis

In essence graft versus host disease in all its forms stems from an attack on host cells by T lymphocytes in the donor

marrow graft. The nature and intensity of this onslaught are determined mainly by three interrelated factors which are difficult to disentangle. Firstly, the character and distribution of the host antigens which provoke this attack are complex and probably vary in different genetic combinations of donor and host. Secondly, graft versus host disease itself reinforces the immunosuppression induced by preparative drug treatment. This immunosuppression interferes with conventional antimicrobial immunity but, paradoxically, it may in addition exaggerate the proliferation of B cells by ablating immunoregulatory lymphocyte populations. Thirdly, opportunistic infections not only threaten these immunocompromised patients directly, but also some viruses, notably cytomegalovirus, may initiate further T cell attacks against infected cells thereby provoking additional immunopathological damage.

Extensive studies in mice with experimental graft versus host disease have thrown considerable light on the genetic factors.⁹ In some combinations of donor and host acute graft versus host is marked by sequential activation of helper T and suppressor T lymphocytes of donor origin. Helper cells fuel the initial immune onslaught, whereas the suppressor cells later modify this process, albeit at the price of impeding the generation of effective antimicrobial immunity. In some mice, however, the subsequent loss of suppressor cells provokes a chronic phase of immunoproliferative activity. In other combinations donor suppressor cells fail to develop, and the clinical picture is dominated throughout by chronic immunoproliferative changes resembling systemic lupus erythematosus. Though there are obvious dangers in extrapolating too literally from experiments on mice to man these observations point to the genetic factors which may determine the clinical outcome.

Judged by the results of laboratory work on the responses of lymphocytes to mitogens, the absolute and relative numbers of blood lymphocyte subpopulations displaying distinctive OKT antigens as markers, and skin test reactions, most patients remain at least partially immunosuppressed for 300-400 days after grafting.¹⁰⁻¹¹ Indeed, a return to normal immunological responsiveness may be delayed for up to four years and is particularly retarded in patients who have suffered either acute or chronic graft versus host disease.¹²⁻¹³ Defective natural killer cell function has also been recorded.¹⁴ These deficiencies largely account for the high risk of opportunistic infections. In addition, decreased numbers in the blood of helper T lymphocytes and increased numbers of suppressor T lymphocytes are regularly observed, providing additional evidence for the immunoregulatory abnormalities which occur after grafting.³⁻¹³ There may also be subtle disturbances in the control of immune responses in tissues primarily affected by graft versus host disease. For example, the antigen presenting cells normally present in skin (Langerhans cells) are reduced in number and impaired in function after marrow transplantation.¹⁵ Yet in animals with graft versus host disease skin keratinocytes acquire the Ia antigens normally associated predominantly with immunologically functional cells.¹⁶

Opportunistic infections are a constant threat to the recipients of marrow transplants and the risks are intensified by the more profound immunosuppression consequent on graft versus host disease.⁴ The agents presenting a particular hazard differ at various stages after transplantation; patients with graft versus host disease are especially vulnerable to infections with cytomegalovirus, adenovirus, and *Pneumocystis carinii*.² Indeed, cytomegalovirus infections may possibly be directly responsible for imbalance in lymphocyte

subpopulations and resulting immunoregulatory disturbances. A Dutch group has noted that while such imbalances develop in recipients of autologous as well as allogeneic bone marrow transplants these may be prevented if care is taken not to transfuse blood products carrying cytomegalovirus into recipients previously free from infection.¹⁷ The frequency with which prolonged cytomegalovirus viraemia follows transplantation of the marrow has been emphasised,¹⁸ and this virus may induce both immunosuppression and autoantibody production in experimental murine and natural human infections.¹⁹⁻²⁰

Prevention

These factors have an important bearing on devising measures likely to prevent or reverse graft versus host disease. Obviously the simplest means of protection is to develop forms of treatment which avoid the need for marrow transplantation altogether. Marrow aplasia is a good example of this therapeutic dilemma. Undoubtedly marrow grafting has improved the outlook for patients with this disorder, which carries a 75% mortality with supportive care alone. The measures taken to reduce the risk of graft rejection, however, have also increased the risks of opportunistic infections and graft versus host disease.² By contrast, intensive immunosuppression and notably antilymphocyte globulin have induced recovery of the bone marrow without resorting to transplantation.²¹ Since many factors such as age and associated disease affect the outcome with any form of treatment comparative trials of different regimens are mandatory—and are now appearing.²²

Another way of avoiding the risks of graft versus host disease implicit in allogeneic grafts is to increase the scope for autologous transplantation. For example, the leukaemic cells may be eliminated with chemotherapy and bone marrow function may then be restored with the patient's own stored marrow cells—provided that the graft can be cleared of leukaemic cells. Monoclonal antibodies reacting specifically with leukaemic cells are one possible approach which is being explored.²³ Most programmes remain dependent on allogeneic marrow grafts, however, and there is intense interest in procedures for selectively removing the T lymphocytes which initiate graft versus host disease while preserving pluripotential stem cells. (The lymphocyte progeny of the grafts are thought to be made tolerant to host antigens.) Some methods such as counterflow centrifugation exploit physical differences among cell types.²⁴ Other investigators have explored the possibility that a period of laboratory culture might allow the selective survival of progenitor cell populations.²⁵ More specific means of removing T lymphocytes have been subjected to clinical trial. One group has achieved this by agglutinating these cells with soybean or forming rosettes with sheep red cells.²⁶ Several laboratories have devised techniques based on monoclonal antibodies specific for T lymphocytes either singly,²⁷ in combination,²⁸ or by linking the antibody to the toxin ricin.²⁹⁻³⁰ All these procedures are technically impressive and clinically promising in terms of avoiding graft versus host disease, but few reports have appeared describing reasonable numbers of patients followed up for adequate periods of time.

The prospects for preventing graft versus host disease by immunosuppressive treatment immediately after grafting have been greatly improved by the introduction of cyclosporin A.³¹ The side effects of this drug still cause concern. Cyclosporin A causes gingival hyperplasia and hypertrichosis

in the recipients of bone marrow.³² As in renal transplantation the drug is nephrotoxic, and the renal damage may prove irreversible.³³ The oncogenic potential of cyclosporin A may have been exaggerated since the lymphoproliferative changes observed in some recipients of organ transplants commonly remit when the drug is withdrawn.³⁴ Nevertheless, the long term risks of malignant disease are unknown, particularly with respect to lymphomas induced by Epstein-Barr virus. Contrary to earlier reports cyclosporin A does not interfere with T lymphocyte activation.³⁵ Nor does immunological competence return earlier in patients treated with this drug as prophylaxis against graft versus host disease than in patients receiving methotrexate for this purpose.³⁶ Thus the risks of infection are not reduced on this score.

Treatment

Established acute graft versus host disease is difficult to reverse. It is particularly responsive to high dose corticosteroids, alone³⁷ or in combination with antilymphocyte globulin³⁸ or methotrexate, but overall mortality has been little reduced. Neither cyclosporin A nor selective monoclonal antilymphocyte antibodies have been properly assessed in these circumstances.² Immunosuppressive drugs, particularly corticosteroids and azathioprine, arrest the progression of chronic graft versus host disease in one third of patients, but a quarter of patients die from infections.⁴ The

scleroderma like features are said to respond to penicillamine.³⁹

Graft versus host disease is one of the most important obstacles to safe, reliable transplantation programmes. Careful, long term trials of competing regimens are essential and should be carried out by units handling adequate numbers of patients. Only trials of this nature will show the extent of unexpected problems, such as the development of myasthenia gravis in one marrow recipient mediated by the donor cells.⁴⁰ Continuing analysis of the cellular mechanisms which induce this complication should lead to more subtle methods of avoiding it. Possibly some T lymphocytes transferred in the marrow or maturing after grafting react with host antigens; others may react with cells infected with viruses, and yet other populations with abnormally proliferating or frankly leukaemic cells.²⁴ Chimerism may persist more commonly than has been suspected, the surviving host haemopoietic cells introducing further complexities in the interpretation of events.⁴¹ Techniques are available for identifying and deleting lymphocytes specifically mediating graft versus host disease and of encouraging the proliferation of populations which benefit the host. The therapeutic value of such advances may prove relevant to a much wider range of disorders.

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- Dinsmore R, Kirkpatrick D, Flomenberg N, *et al*. Allogeneic bone marrow transplantation for patients with acute nonlymphocytic leukemia. *Blood* 1984;63:649-56.
- O'Reilly RJ. Allogeneic bone marrow transplantation: current status and future directions. *Blood* 1983;62:941-84.
- Stastny P, Stemberger VA, Vischer T, Ziff M. Homologous disease in the adult rat, a model for autoimmune disease. II. Findings in the joints, heart and other tissues. *J Exp Med* 1965;122:681-92.
- Deeg HJ, Storb R, Thomas ED. Bone marrow transplantation: a review of delayed complications. *Br J Haematol* 1984;57:185-208.
- Sullivan KM, Deeg HJ, Sanders JE, *et al*. Late complications after marrow transplantation. *Semin Hematol* 1984;21:53-63.
- Ralph DD, Springmeyer SC, Sullivan KM, Hackman RC, Storb R, Thomas ED. Rapidly progressive air-flow obstruction in marrow transplant recipients. Possible association between obliterative bronchiolitis and chronic graft-versus-host disease. *Am Rev Respir Dis* 1984;129:641-4.
- Graham-Brown RAC, Sarkany I. Scleroderma-like changes due to chronic graft-versus-host disease. *Clin Exp Dermatol* 1983;8:531-8.
- Snover DC. Acute and chronic graft-versus-host disease. Histopathological evidence for two distinct pathogenetic mechanisms. *Hum Pathol* 1984;15:202-5.
- Pals ST, Radaszkiewicz T, Gleichmann E. Allosuppressor (and allohelper) T cells in acute and chronic graft-versus-host disease. IV. Activation of donor allosuppressor cells is confined to acute GVHD. *J Immunol* 1984;132:1669-78.
- Ueda M, Harada M, Shiobara S, *et al*. T lymphocyte reconstitution in long-term survivors after allogeneic and autologous marrow transplantation. *Transplantation* 1984;37:552-6.
- Witherspoon RP, Lum LG, Storb R. Immunologic reconstitution after human marrow grafting. *Sem Hematol* 1984;21:2-10.
- Witherspoon RP, Matthews D, Storb R, *et al*. Recovery of in vivo cellular immunity after human marrow grafting. Influence of time postgrafting and acute graft-versus-host disease. *Transplantation* 1984;37:145-50.
- Tsai M-S, Dobbs S, Brkic S, Ramberg R, Thomas ED, Storb R. Cellular interactions in marrow-grafted patients. II. Normal monocyte antigen-presenting and defective T-cell-proliferative functions early after grafting and during chronic graft-versus-host disease. *Transplantation* 1984;37:556-61.
- Rook AH, Frederick WRJ, Burns WH, *et al*. Natural killer cell activity and interferon release predict survival from viral infection in bone marrow transplant recipients. *Transplant Proc* 1983;15:1773-6.
- Perreault C, Pelletier M, Landry D, Gyger M. Study of Langerhans cells after allogeneic bone marrow transplantation. *Blood* 1984;63:807-11.
- Breathnach SM, Katz SI. Keratinocytes synthesize Ia antigen in acute cutaneous graft-versus-host disease. *J Immunol* 1983;131:2741-5.
- Verdonck LF, de Gast GC. Is cytomegalovirus infection a major cause of T cell alterations after (autologous) bone-marrow transplantation? *Lancet* 1984;ii:932-5.
- Zaia JA, Forman SJ, Gallagher MT, Vanderwal-Urbina E, Blume KG. Prolonged human cytomegalovirus viremia following bone marrow transplantation. *Transplantation* 1984;37:315-7.
- Grundy JE, Shearer GM. The effect of cytomegalovirus infection on the host response to foreign and hapten-modified self histocompatibility antigens. *Transplantation* 1984;37:484-90.
- Villar LA, Massanari RM, Mitros FA. Cytomegalovirus infection with acute erosive esophagitis. *Am J Med* 1984;76:924-8.
- Champion R, Ho W, Gale RP. Antithymocyte globulin treatment in patients with aplastic anemia. A randomized trial. *N Engl J Med* 1983;308:113-8.
- Camitta B, O'Reilly RJ, Sensenbrenner L, *et al*. Antithoracic duct lymphocyte globulin therapy of severe aplastic anemia. *Blood* 1983;62:883-8.
- Falkenberg JHF, Jansen J, van der Vaart-Duinkerken N, *et al*. Polymorphic and monomorphic HLA-DR determinants on human hematopoietic progenitor cells. *Blood* 1984;63:1125-32.
- Witte T de, Raymakers R, Plas A, Koekman E, Wessels H, Haanen C. Bone marrow repopulation capacity after transplantation of lymphocyte-depleted allogeneic bone marrow using counterflow centrifugation. *Transplantation* 1984;37:151-5.
- Mauch P, Lipton JM, Hamilton B, *et al*. Lethal graft-versus-host disease: modification with allogeneic cultured cells. *Blood* 1984;63:1112-9.
- Friedrich W, Vetter U, Heymer B, *et al*. Immunoreconstitution in severe combined immunodeficiency after transplantation of HLA-haplo-identical, T-cell-depleted bone marrow. *Lancet* 1984;ii:761-4.
- Hale G, Bright S, Chumbley G, *et al*. Removal of T cells from bone marrow for transplantation: a monoclonal antilymphocyte antibody that fixes human complement. *Blood* 1983;62:873-82.
- Prentice HG, Janossy G, Price-Jones L, *et al*. Depletion of T lymphocytes in donor marrow prevents significant graft-versus-host disease in matched allogeneic leukaemic marrow transplant recipients. *Lancet* 1984;ii:472-6.
- Filipovich AH, Youle RJ, Neville DM Jr, Vallera DA, Quinones RR, Kersey JH. Ex-vivo treatment of donor bone marrow with anti-T-cell immunotoxins for prevention of graft-versus-host disease. *Lancet* 1984;ii:469-71.
- Guinones RR, Youle RJ, Kersey JH, *et al*. Anti-T-cell monoclonal antibodies conjugated to ricin as potential reagents for human GVHD prophylaxis: effect on the generation of cytotoxic T cells in both peripheral blood and bone marrow. *J Immunol* 1984;132:678-83.
- Powles RL, Clink HM, Spence D, *et al*. Cyclosporin A to prevent graft-versus-host disease in man after allogeneic bone marrow transplantation. *Lancet* 1980;ii:327-9.
- Harper JJ, Kendra JR, Desai S, Staughton RCD, Barrett AJ, Hobbs JR. Dermatological aspects of the use of cyclosporin A for prophylaxis of graft-versus-host disease. *Br J Dermatol* 1984;110:469-74.
- Merion RM, White DJG, Thiru S, Evans DV, Calne RY. Cyclosporin: five years' experience in cadaveric renal transplantation. *N Engl J Med* 1984;310:148-53.
- Starzl TE, Porter KA, Iwatsuki S, *et al*. Reversibility of lymphomas and lymphoproliferative lesions developing under cyclosporin-steroid therapy. *Lancet* 1984;ii:583-7.
- Forman SJ, Gallagher MT, Zaia JA, Wright C, Blume KG. Cyclosporin does not prevent in vivo expression of T cell activation antigens following allogeneic bone marrow transplantation. *Transplantation* 1984;37:19-20.
- Witherspoon RP, Deeg HJ, Lum LG, *et al*. Immunologic recovery in human marrow graft recipients given cyclosporin or methotrexate for the prevention of graft-versus-host disease. *Transplantation* 1984;37:456-61.
- Kanojia MD, Anagnostou AA, Zander AR, *et al*. High-dose methylprednisolone treatment for acute graft-versus-host disease after bone marrow transplantation in adults. *Transplantation* 1984;37:246-9.
- Doney KC, Weiden PL, Storb R, Thomas ED. Treatment of graft-versus-host disease in human allogeneic marrow graft recipients. A randomized trial comparing antithymocyte globulin and corticosteroids. *Am J Hematol* 1981;11:1-8.
- Summerfield GP, Bellingham AJ, Bunch C, Woodrow JC. Successful treatment of chronic cutaneous graft-versus-host disease (GVHD) with penicillamine. *Clin Lab Haematol* 1983;5:313-8.
- Smith CI, Aarli JA, Biberfeld P, *et al*. Myasthenia gravis after bone marrow transplantation. Evidence for a donor origin. *N Engl J Med* 1983;308:1565-8.
- Singer JW, Keating A, Ramberg R, *et al*. Long-term stable hematopoietic chimerism following marrow transplantation for acute lymphoblastic leukemia: a case report with in vitro marrow culture studies. *Blood* 1983;62:869-72.