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requirement for immune suppression or risk of GVHD. However, as the harvested stem cells may be contaminated with a low level of malignant cells, there is a risk of relapse. The relapse risk depends on the underlying disease and response to pretransplantation chemo/radiotherapy. Studies attempting to purge the collected stem cells of contaminating malignant cells have been performed, but have failed to show any improvements in overall survival, relapse rate, or disease-free survival compared to using unmanipulated stem cells. Allogeneic haemopoietic stem cell transplantation Selection of donors Fully HLA-matched sibling donors are only available for about one in three recipients. Minor disparity between the HLA types of donor and recipient are allowable, but the greater the disparity, the higher the frequency of complications. For those without such a donor, possible sources are (1) volunteer donor banks, which can provide acceptably HLA-matched donors for about 80% of recipients with the same genetic disequilibrium as the donor pool; (2) umbilical cord blood banks; and (3) haploidentical family donors. Conditioning regimen Conditioning regimens include measures to induce immunosup- pression (required for all allogeneic transplants, excepting those from identical twin donors) and, when appropriate, eradicate dis-eased bone marrow. They may be (1) myeloablative (no autolo- gous recovery possible)—cyclophosphamide with (a) total body irradiation (TBI), or (b) busulphan, or (c) antilymphocyte globulin; or (2) nonmyeloablative—fludarabine with varying combinations of (a) antilymphocyte globulin or alemtuzumab (anti-CD52); (b) low- dose cyclophosphamide, melphalan, or busulphan; and (c) low-dose TBI. After transplantation, donor lymphocyte infusions may be given to patients with malignant disorders to enhance the graft-versus-leu- kaemia or graft-versus-tumour effect. Graft-versus-host disease Acute GVHD—this major cause of transplant-related morbidity and mortality may develop at any time within the first 6 weeks after trans-plantation. The clinicopathological manifestations are secondary to the recognition of alloantigens in recipient tissue, by donor-derived T lymphocytes, hence the importance of HLA typing. Manifestations involve (1) skin—maculopapular rash, generalized erythroderma, desquamation, and bullae; (2) liver—severity graded according to level of serum bilirubin; and (3) gut—diarrhoea, persistent nausea, and pain/ileus. Chronic GVHD—may follow acute GVHD or emerge de novo sev-eral months after transplantation. Mainly affects the skin, but al- most any organ may be affected, for example, lung (bronchiolitis obliterans), gut, liver, eyes (sicca syndrome), buccal mucosa, skin (sclerodermatous changes), and musculoskeletal system. Chronic GVHD parallels graft-versus-leukaemia effect and total suppression is associated with increased relapse in some leukaemia or lymphoma transplants. Prevention/treatment—these present a major challenge. Standard prevention is with ciclosporin, with or without methotrexate and/or antilymphocyte globulin. Initial treatment of both acute and chronic GVHD is with high-dose steroids. Other complications and prognosis Conditioning regimens have considerable toxicity. (1) Acute—the pa- tient is particularly vulnerable to infectious complications in a period of intense neutropenia, usually lasting 2 to 4 weeks after transplant- ation and lymphopenia, which may last some months. Patients are managed in isolation facilities, with (as appropriate) prophylactic measures against fungal infection, herpes simplex, Gram-negative bacterial infection, and Pneumocystis jirovecii. Prophylactic regimens vary according to local experience with resistant organisms and availability of newer antimicrobials. Broad-spectrum intravenous antimicrobial therapy is used to treat fevers empirically. Ganciclovir is given if routine monitoring shows evidence of cytomegalovirus reactivation. (2) Chronic—common manifestations include retard- ation of growth (particularly if transplanted in childhood), endocrine impairment, infertility, intellectual impairment (following cranial irradiation).

 Prognosis—once the graft is fully established and tolerance is re- constituted, immunosuppression may be stopped. In the absence of GVHD, immune suppression can be weaned from as early as 3 months after transplantation. The procedure offers hope to many patients with life-threatening marrow failure or malignant disease for which no other treatment is available, but in the long term recipients have a reduced life expectancy due to relapse of the underlying dis- ease, infection, and chronic GVHD. There is a small increased risk of second malignancies. Introduction The idea that haemopoietic stem cells (HSCs) from the bone marrow could be transferred from a normal individual to a pa- tient to replace defective bone marrow has a long history. With the exception of rare instances where marrow was obtained from an identical twin, such attempts in humans universally failed until an understanding of the immune processes involved in tolerance and rejection became available. Much of the pioneering work in making possible human bone marrow transplantation was carried out by E. Donnall Thomas and colleagues in the United States of America, work for which Thomas received the Nobel Prize jointly in 1990. Experiments on inbred mice had shown that lethally ir- radiated animals could be rescued by intravenous transfusion of bone marrow from unirradiated mice and that this protection was the result of engraftment of the normal marrow in the re- cipient. Successful engraftment depended upon the donor marrow being genetically acceptable by the recipient mouse or the re- cipient mouse being sufficiently immunosuppressed. Engraftment when there was immunological disparity between the donor and recipient was followed after a period of 2 weeks or so by a ‘sec- ondary’ disease in which the recipient mouse failed to thrive and developed gastrointestinal disorders, liver failure, and skin disease, leading to poor further development and eventual death from in- fection. This so-called runt disease is the murine equivalent of graft-versus-host disease (GVHD) in humans, in which immuno- competent cells from the immunologically disparate donor mount an attack against recipient tissues. From these and other experi- ments in outbred animals it was recognized that transplantation of bone marrow would carry the special risk of GVHD and that histocompatibility would be a critical requirement for successful transplantation. Furthermore, considerable immunosuppression would be required to achieve engraftment in all transplants except those from syngeneic (identical twin) donors. However, it was also recognized that not only the haemopoietic but also the immune system would be replaced (reconstituted from donor stem cells) following myeloablation and that the need for immunosuppres- sion would cease, except for the management of GVHD, once donor immune reconstitution was complete. Transplants in dogs demonstrated that total-body irradiation and cyclophosphamide were sufficiently immunosuppressive to permit engraftment, and that GVHD could be controlled to some extent with methotrexate, if there was not great disparity between the histocompatibility antigens of donor and recipient. The elu- cidation of the major histocompatibility complex (MHC) on chromosome 6 in humans, with the identification of the histocom- patibility antigens at the A, B, or C (class I) and DR (class II) loci of the HLA system, finally allowed the identification of appropriate donors for human transplantation. The paramount importance of histocompatibility in haemopoietic stem cell transplantation (HSCT) has been confirmed subsequently by extensive clinical practice. The first successful transplant from a nonidentical, but HLA-compatible, sibling was carried out in 1968 for a patient with severe combined immune deficiency where the underlying dis- ease prevented rejection. Further successful allogeneic transplants from sibling donors using conditioning with total-body irradiation and cyclophosphamide carried out in 1969 in Seattle (Washington, United States of America) by the group led by Thomas. Many thousands of such transplants have been carried out subsequently, though the precise indications and timing for transplant (during the disease course) particularly in malignant disease, are not al- ways as clear as they might be (Table

22.8.2.1) and the problems of GVHD, graft failure, and infection remain hazards which con- tribute to transplant-related mortality. Allogeneic HSCT must al- ways be compared with best available alternative therapies. On the other hand, better support with blood products and antibiotics, improved tissue typing techniques, and the introduction of less toxic methods of delivering conditioning to control rejection and Table 22.8.2.1 Main disorders for which haemopoietic stem cell transplantation may be appropriatea Acquired disorders Haematological malignancies Acute leukaemias Chronic myeloid leukaemia Non-Hodgkin lymphoma (including CLL) Hodgkin lymphoma Myeloma and other plasma cell dyscrasias Solid tumours Although HSCT has been used as adjunct to chemotherapy in solid tumours, results to date
are universally disappointing Bone marrow failure syndromes Myelodysplastic syndromes Myeloproliferative neoplasms Aplastic anaemia Paroxysmal nocturnal haemoglobinuria Congenital disorders Haematological Fanconi anaemia β-thalassaemias (an increasingly important disorder for considering HSCT) Diamond–Blackfan anaemia Kostmann syndrome Immunological Severe combined immune deficiency and other primary immune deficiencies Chronic granulomatous disease Metabolic Malignant osteopetrosis Lysosomal storage diseases CLL, chronic lymphocytic leukaemia. a Stem cell transplantation may be considered an option according to availability of a suitable donor, the stage or severity of the disease, and the availability and effectiveness of other forms of management.

22.8.2 Haemopoietic stem cell transplantation 5581 GVHD, as well as better selection of recipients, have improved out- comes steadily over the last 40 years. Histocompatibility complex and haemopoietic stem cell transplantation The organization of the MHC on chromosome 6, and its importance in transplantation, is described in detail in Chapter 4.7. The close- ness of the relevant genes in the complex means that within families there is little crossing-over in germ-line cells and inheritance more or less follows the autosomal pattern, so that the chances of a sibling having the same HLA type as a patient is about one in four. This is genotypic identity in which not only the HLA types but also many unidentified sequences in the MHC are identical. At each HLA locus there are large numbers of possible alleles in humans leading to a potential of many millions of different histocompatibility profiles. However, within populations, certain HLA alleles tend to be asso- ciated and segregate together, ‘genetic disequilibrium’, so that it is theoretically and practically possible to find phenotypically identical pairs within an unrelated population. The identification of phenotypes was originally based upon sero- logical testing for A, B, and DR antigens. The introduction of mo- lecular techniques for identifying DNA sequences directly has shown that there may be a large number of HLA gene products whose cog- nate protein molecules are assigned to the same phenotype by sero- logical methods. HLA typing of individuals within populations has made possible the creation of large volunteer donor panels of individ- uals prepared to supply HSCs. However, matching the MHC between unrelated pairs even by molecular techniques gives at best pheno- typic identity and there are likely to be many fine genetic differences. Selection of donors by improved typing techniques has reduced the risks associated with unrelated transplants but restricted the range of appropriate donors. It has also become clear that there are very wide variations in the linkage disequilibria at MHC loci between different populations of the world so that a donor panel of one ethnic type may have much reduced chances of providing stem cells for another. Where there is an HLA disparity between donor and recipient, HSCT is possible, but the incidence of complications rises steadily as the degree of disparity increases. It is also apparent that the major antigens of the MHC (HLA class I and II antigens) are not the only ones important in determining the incidence and severity of GVHD. GVHD is mediated by donor-derived CD4+ helper

and CD8+ cyto- toxic T lymphocytes. These T cells recognize allogeneic MHC mol- ecules and minor histocompatibility antigens (self proteins where a polymorphism exists between donor and recipient) expressed on normal recipient tissues. The role of specific major and minor re- cipient antigens in the pathogenesis of GVHD is only now being worked out in any detail. As discussed later, the cell-mediated im- mune attack on normal tissues causing GVHD is also linked to an ability to attack abnormal, particularly malignant cells, producing a graft-versus-leukaemia/lymphoma (GVL) effect. Much effort has been directed at identifying the donor cells, which mediate the GVL effect and the antigen-presenting cells of the recipient, which facili- tate the GVL effect in order to separate GVL from GVHD, so far with inconclusive results. It appears that many target antigens of GVL and GVHD are shared. The problems and benefits of immunological disparity obviously only apply in the allogeneic transplantation setting and are absent when autologous stem cells are used to restore haemopoiesis after intensive chemotherapy. Haemopoietic stem cells Stem cells are defined by their ability to proliferate and differentiate into one or more specific cell lineages and also to maintain the stem cell pool by self-renewal. HSCs give rise to the blood cell lineages— red cells, granulocytes, and platelets as well as the cells of the im- mune system. It seems probable that a single stem cell can repopulate the blood and immune systems of an entire animal. HSCs can be identified by immunophenotyping and their ability to repopulate marrow. The best in vitro techniques have suggested that the human HSC is closely related to precursors that carry an antigen designated CD34, lack other haemopoietic markers including CD33, and have no lineage-specific markers. Whether such cells are truly the most primitive cells that are capable of giving rise to both haemopoietic and immunological precursors is not of practical importance since successful haemopoietic reconstitution, both in allogeneic and au- tologous transplants, is closely related to the number of such cells present in the donation. The CD34+CD33– cells represent some 1 × 10–3 to 10–4 of the cells of normal human haemopoietic marrow. Sources of haemopoietic stem cells HSCs develop in specific sites within the bone marrow, designated niches, which include specialized cells of the bone marrow stroma. The stem cell is not fixed in its environment, and may leave the marrow, enter the circulation, and home once again to the marrow or to other sites depending on the chemokine and cytokine signals it receives (see Chapter 22.2.1). Small numbers of CD34+ HSCs cir- culate in normal blood, and this number is greatly increased during the marrow recovery after cytotoxic chemotherapy. Administration of certain cytokines, particularly granulocyte colony-stimulating factor (G-CSF), increases the number of circulating CD34+ cells enor- mously, such that for a period of a few days following treatment there are adequate numbers in the circulation to use as a source of cells for transplantation. Homing and mobilization, and recirculation of HSCs is a continuous, dynamic process—even under normal conditions. In the early development of the fetus, haemopoiesis takes place in the liver. Fetal liver cells have been used as a source of HSCs, mainly for the treatment of inherited severe combined immune deficiency. The logistics of such transplants, which require 11-week-old fetal livers, make this an impractical approach. However, research on embryonic stem cells suggests that there may be other important sources of stem cells, not only for haemopoiesis but for other types of tissue replacement. Of more immediate practical importance was the finding that umbilical cord blood (UCB) contained large num- bers of cells with high proliferative potential and characteristics of stem cells. UCB has become a third practical source of donor cells. Each of these sources—bone marrow, peripheral blood, and cord blood—has advantages and disadvantages that impinge on clinical management. A critical requirement for successful transplantation is that there should be a sufficient number of stem cells. The ability to expand stem cells ex vivo would solve this and other requirements, but so far this has not proved to be useful clinically.

section 22 Haematological disorders 5582 HSCs from bone marrow Until about 1993, most transplants were conducted using bone marrow stem cells, but peripheral blood mobilized HSCs are now the preferred option. Much of the data concerning the success and prob- lems of stem cell transplantation are derived from the use of bone marrow. Bone marrow is harvested with the patient/donor under general anaesthetic by aspiration from the posterior and superior iliac crests, and if necessary the sternum. Experience has shown that some 3 × 108 nucleated cells/kg recipient body weight are required for successful engraftment and this usually involves collecting 1 to 1.5 litres of bone marrow (mixed, of course, with blood). Donors may have a unit of blood collected before harvesting, which is re- turned at the end of the procedure to ameliorate the anaemia. The procedure takes approximately 1 h and the donor usually requires brief admission to hospital to recover. Serious complications are ex- tremely rare and are those associated with the general anaesthetic or local complications such as osteomyelitis or abscess formation. The advantage of this source of stem cells from the donors' viewpoint is that collection is rapid, with a maximum of 48 h involvement. The disadvantage is the need to be admitted to hospital for a general an- aesthetic and the pain or discomfort and anaemia that follow the procedure. HSCs from peripheral blood HSCs may be mobilized into the peripheral blood following ex- posure to G-CSF. For allogeneic transplantation, donors receive G-CSF (filgrastim or lenograstim) at a dose of 10 μg/kg subcuta- neously daily for 5 days. The peripheral granulocyte count rises to 30 × 109/litre or higher and CD34+ cells appear in the peripheral blood reaching a maximum 5 to 6 days after the start of treatment. Leucocytes are collected by leucapheresis with the objective of reaching more than 2 × 106 CD34+ cells/kg body weight of the re- cipient. Sufficient cells can usually be collected from normal donors in one procedure, although poor mobilizers are found in the normal population and are not infrequent when autologous stem cell collec- tion from patients is required following chemotherapy. Plerixafor, an inhibitor of the CXCL12/CXCR4 axis, which retains stem cells in the haemopoietic niche, is an important adjunct to G-CSF in mo- bilization and has the advantage of a more rapid effect. The main disadvantage for donors of this type of stem cell collection is that of bone pain or ache following the injections of G-CSF and the procedure of leucapheresis. Rare instances of splenic rupture have been recorded. There has been some concern about the theoretical potential of G-CSF to cause cytogenetic abnormalities that could lead to leukaemia; although no such effect has yet been found in normal donors given the cytokine, long-term follow-up is no longer mandated by donor registries. The main advantages are the avoid- ance of admission to hospital and a general anaesthetic. When au- tologous collection of stem cells is required, the concentration of CD34+ cells may be increased further by giving cyclophosphamide (or some other chemotherapeutic agents, such as etoposide) before starting the G-CSF. Autologous HSCT is used mainly for patients with malignant disease, for marrow rescue following further inten- sive chemotherapy. The use of peripheral blood for harvesting stem cells for allogeneic transplants provides high numbers of CD34+ cells and more rapid engraftment than with bone marrow-derived stem cells. On the other hand, peripheral blood contains more T cells and, although original concerns that acute GVHD (aGVHD) would be unaccept- ably severe unless T cells were removed have proved unfounded, chronic GVHD (cGVHD), especially extensive disease, is, more prevalent than in bone marrow and UCB transplants and is the main nonrelapse cause of mortality of peripheral blood stem cell trans- plants. Nevertheless, the ease of collection and advantages of rapid engraftment have meant that most autologous transplants (where GVHD is not a problem), and the majority of allogeneic transplants, are currently sourced from the peripheral blood. HSCs from umbilical cord blood Sourcing HSCs from UCB has several theoretical and practical advantages. UCB is widely available with no risk to mother or in- fant donor, there is

low viral contamination, the immaturity of the immune cells may theoretically reduce the risk of GVHD, and the cells may readily be stored frozen and made available rapidly for urgent transplantation. Furthermore, a balance of UCB stem cells from different ethnic groups to take advantage of genetic disequi- librium can be achieved and specific HLA types can be targeted. A disadvantage is the relatively small volume of UCB and hence low total numbers of HSCs. UCB donations have mostly been used to transplant children. Recently the use of double or multiple UCB do- nations to increase the infused HSC dose has been introduced for adult transplants; further follow-up is needed to assess the results, though there is early evidence that the risk of cGVHD is less than with bone marrow or peripheral blood donations. The storage and administrative costs of UCB are high compared with sourcing from unrelated donors at the time of transplant. A further difficulty is the lack of any back-up source of cells should the graft fail or disease relapse occur. For adults with impaired thymic function the lack of memory T lymphocytes specific for common latent viruses such as cytomegalovirus (CMV) in the donation may result in repeated viral reactivation. Nevertheless, UCB transplants are increasing as a prac- tical source of HSCs in the future. Plasticity of stem cells The bone marrow and other tissues contain totipotent stem cells. These bone marrow-derived cells may differentiate to cardiac muscle cells, nerve cells, striated muscle fibres, and many other tis- sues, whether they are ectodermal, mesodermal, or endodermal in origin. This potential is also present in embryonic stem cells. HSCs have been used in phase I/II studies examining the effect on cardiac function after myocardial function and other insults. Although it is possible to identify HSCs in the myocardium after such proced- ures, practical benefits have not yet been clearly revealed. A second class of bone marrow cells which have stem cell properties in vitro and may be stem cells in vivo are the mesenchymal stromal (stem) cells. In vitro they can differentiate into a variety of mesodermal tis- sues. Their potential interest for HSC transplantations lies in their immunomodulatory effects. Early clinical trials suggest they may be able to modify aGVHD. Donors for allogeneic stem cell transplantation Problems of transplant-related morbidity and mortality, graft rejec- tion, GVHD, and infection increase with increasing donor disparity.

22.8.2 Haemopoietic stem cell transplantation 5583 HLA-matched sibling donors are not only phenotypically matched for the MHC, but have genotypic identity throughout most of the MHC. This does not eliminate transplant-related morbidity and mortality, but reduces the incidence and severity of the problems compared with unrelated volunteer donors matched only pheno- typically for the MHC and mismatched at minor histocompatibility antigens. HLA-matched sibling donors are only available for about one in three recipients in populations with an average of two or three children per family. To overcome this shortfall, volunteer donor banks have been established, now including more than 10 million typed donors worldwide. These panels can provide HLA-suitable matches for about 80% of recipients with the same genetic disequi- librium as the donor pool, though finding the right match may take several weeks. Extensive immunosuppression of the recipient is re- quired prior to transplantation to prevent graft rejection and after transplantation to control GVHD. New methods of immunosup- pression which allow the stepwise engraftment of donor marrow may produce a greater degree of tolerance and permit successful transplantation of HSCs with some degree of HLA disparity. UCB banks have been established in many countries. Their use is likely to increase if the efficacy of double or multiple donations remain ef- fective for adults after longer-term follow-up. An advantage of using haploidentical donors (typically parents or a child) is their ready availability. Management of transplant recipients Conditioning regimen The treatment of recipients prior to transplantation includes meas- ures to induce

immunosuppression and eradicate diseased bone marrow. In HSC transplantation for malignant disease, most proto- cols to date have contained cyclophosphamide combined either with total-body irradiation, single dose or fractionated, or with alkylating agents such as busulphan. For nonmalignant conditions irradiation should be avoided. For acquired aplastic anaemia, cyclophospha- mide, either alone or combined with antithymocyte globulin, has been the major conditioning regimen. Some of the more widely used regimens are indicated in Table 22.8.2.2. The incidence and severity of GVHD was reduced by giving methotrexate in a short protocol after transplantation; the introduction of ciclosporin fur- ther improved results. Such conditioning regimens, particularly for malignant and genetic disorders, carry delayed as well as acute toxicity, particularly for children. Where radiation is used (and to a lesser extent busulphan), infertility is usual, growth is retarded, and other endocrine functions may be impaired. Where transplantation is used for patients who have already received irradiation or chemo- therapy to the central nervous system, for example, patients with a relapsed acute lymphoblastic leukaemia, intellectual impairment as well as the earlier-mentioned problems are common. Success of stem cell transplantation in certain malignant condi- tions is related to the cellular immune response to recipient cells and tissue (GVL), provided by donor lymphocytes, rather than the direct cytotoxic effect of conditioning. Repopulation of marrow by donor HSCs does not require the immediate abolition of recipient marrow. Full donor chimerism may be achieved over time rather than in one step, and in some instances mixed stable chimerism of both donor and recipient cells may occur. Conditioning regimens have been introduced which do not rely on cytotoxic measures to obliterate recipient marrow and immune system, but which have increased immunosuppressive action. Such regimens include fludarabine, a highly immunosuppressive drug that is not very cytotoxic, often combined with antithymocyte globulin, monoclonal antibodies (e.g. alemtuzumab, anti-CD52), and/or low-dose total-body irradi- ation. Removal of T lymphocytes from the donor preparation (T-cell depletion), to reduce aGVHD with subsequent later add-back of donor lymphocytes to reduce the chances of relapse, is also used (Table 22.8.2.2). Results using this approach have been encouraging and reduced-intensity transplantation regimens are widely used for lymphomas and leukaemias, though long-term follow-up is re- quired. The reduced toxicity has allowed the use of HSC transplant- ations for patients up to 60 years of age or even older. Recent modifications to conditioning regimens for recipients of haploidentical donor HSC transplantations have demonstrated that crossing the HLA barrier without unacceptably high inci- dences of graft rejection, severe GVHD, and transplant-related mortality is achievable. This has included the use of high-dose post- transplantation cyclophosphamide to selectively deplete in vivo donor-derived alloreactive T cells during the phase of maximal pro- liferation in order to induce immune tolerance. Graft-versus-host disease GVHD is the consequence of immune attack on recipient tissues by donor lymphocytes. Early experiments on mice showed that the Table 22.8.2.2 Outline of examples of conditioning regimens for allogeneic haemopoietic stem cell transplantation Conditioning regimen Indications Myeloablative Cyclophosphamide at 120 mg/kg + TBI of 750–1400 cGy Acute leukaemia Chronic myeloid leukaemia Relapsed lymphoma Cyclophosphamide at 120 mg/kg + busulphan at 16 mg/kg As above β-thalassaemia major Other congenital bone marrow disorders Cyclophosphamide at 200 mg/kg ± ALG Acquired aplastic anaemia Cyclophosphamide at 25–100 mg/kg + TBI of 200 cGy Fanconi anaemia Reduced-intensity conditioning Fludarabine at 30 mg/m2 Fanconi anaemia ± ALG or alemtuzumab Congenital disorders of haemopoiesis or immune system

- low-dose cyclophosphamide or melphalan or busulphan ± low-dose TBI (200 cGy) Acquired aplastic anaemia With DLIa or virus-specific T cellsb Malignant disorders ALG, antilymphocyte globulin; DLI, donor lymphocyte infusions; TBI, total body irradiation. Lower doses given in single fraction, higher doses fractionated. a Given 3 months or longer post infusion to provide GVL or graft-versus-tumour effect. b Current phase II and III clinical studies are exploring the role of virus-specific T-cell infusions in the management of viral reactivation.

section 22 Haematological disorders 5584 murine equivalent (runt disease) developed when immune com- petent donor lymphocytes were given to immunosuppressed, im- munologically disparate recipients. Skin, gut, and liver were the main organs affected. In human HSCT, mismatch in the MHC be- tween donor and recipient correlates with the severity of GVHD. However, the pathogenesis of GVHD involves more than the im- munological disparity; inflammation, infection, tissue damage, and the gut microbiota play a part in determining the severity of the disease and which organs are affected. The complexity of the pathogenesis has hindered a clear understanding of all the path- ways involved and hence fully effective prevention and treatment. Historically, GVHD was divided into aGVHD which appeared within the first 100 days and cGVHD with a later onset. It is now accepted that both may coexist and that there is overlap in the pathogenesis. From a practical point of view, the distinction re- mains a useful concept. Acute GVHD Originally defined aGVHD, manifest by various degrees of skin, gut, and liver damage (Fig. 22.8.2.1), occurs within the first 3 months or so of the HSCT. For each organ, a score of 0 to 4+ was given, depending on the degree of damage and an overall aGVHD score of grade 0 to IV devised to record overall severity (Table 22.8.2.3). Some two-thirds of patients transplanted from HLA-matched sibling or volunteer donors will experience some de- gree of GVHD—grade III to IV carrying a high risk of transplant- related mortality. Increasing donor–recipient HLA mismatch is associated with higher grades of GVHD but patient age, previous chemotherapy, irradiation, and infections also increase the risk. The basic classification of aGVHD has proven useful in comparing the outcome of various studies of HSCT conditioning in different diseases, but has limitations for understanding the pathogenesis. The National Institutes of Health consensus criteria include ‘late- onset acute GVHD’ an overlap syndrome with cGVHD. A number of steps are involved in the pathogenesis of GVHD. Animal models suggest initiation involves activation of antigen-presenting cells re- lated to underlying disease, chemotherapy, conditioning regimen, and radiation which may release proinflammatory cytokines such as tumour necrosis factor (TNF)-α. This may lead to an interaction with donor T lymphocytes within lymphoid tissues such as Peyer’s (a) (c) (e) (b) (d) Fig. 22.8.2.1 Skin manifestations of acute and chronic GVHD. Acute GVHD: (a) grade I, skin +, showing typical palmar maculopapular rash (recovered); (b) grade IV, skin 4+, generalized erythroderma with early exfoliation; liver 3+, bilirubin greater than 250 μmol/litre (fatal); (c) grade III, skin 4+, bullous desquamation (recovered). Chronic GVHD: (d) sclerotic scarring on back; (e) severe ulceration and contracting scleroderma-like skin involvement.

22.8.2 Haemopoietic stem cell transplantation 5585 patches in the gastrointestinal tract. The reduction of tissue damage in reduced-intensity conditioning regimens may in part explain the reduction in GVHD severity. Tissue damage may also explain the activation of GVHD by virus infections such as CMV. The next and fundamental stage is the activation, differentiation, and pro- liferation of the donor T cells, principally CD4+ and CD8+ cells. A variety of cell types including regulatory T cells, natural killer cells, and mesenchymal stromal cells seem to have modifying roles

in aGVHD in ways that are not well understood but which offer tantalizing clues to improved treatment. The final stage of aGVHD is due to cytotoxic T-cell-mediated attack involving perforin and granzyme pathways as well as other cytokine-mediated inflamma- tory pathways.
Chronic GVHD cGVHD is usually defined as GVHD developing 100 days or more after transplant. Following HSCT, some 40 to 70% of patients de- velop some cGVHD. The pathogenesis is even more complex than that of aGVHD. While skin, liver, and gastrointestinal tract remain principal targets (Fig. 22.8.2.1), other targets such as lung, eyes (Sicca syndrome), mucous membranes, and heart (pericarditis) may be involved. Auto- and alloantibodies are common in cGVHD though their pathogenetic role is not clear. However, it is likely that B cells play a critical role in cGVHD. There is usually a degree of induced immunodeficiency and susceptibility to infection. cGVHD may be transient, persistent, or progressive. Diagnostic criteria for cGVHD have been developed by the National Institutes of Health consensus forum, based on extent of the process and severity of damage. As with aGVHD a viral infection may trigger a relapse. In HSCT for malignant disease, complete absence of cGVHD is associ- ated with an increased risk of relapse, indicating the close relation- ship between GVHD and GVL effect. Prevention of GVHD Prophylaxis has mainly relied on the use of ciclosporin with or without methotrexate. Complete T-cell depletion of the donor graft may prevent GVHD but the increased incidence of graft failure or malignant relapse means there is no overall survival advantage. The polyclonal antithymocyte globulins have been used in addition to ciclosporin after transplantation without clear-cut benefit. Clearly selecting the most appropriate donor possible to ameliorate GVHD is an essential part of the prevention of GVHD. Treatment of GVHD The first approach to management of grade II to IV GVHD is high- dose corticosteroids, usually 1 to 2 mg prednisolone/kg body weight, together with ciclosporin or another calcineurin inhibitor. Topical steroids may be effective in grade I to II skin GVHD. The difficul- ties arise in steroid-unresponsive or relapsing GVHD. About half the patients so treated will achieve complete or partial responses and failure to respond correlates with a poor prognosis. If there is no response within 5 to 15 days or if there is early deterioration on steroids, second-line treatment needs to be started. The large number of agents considered indicates the difficulty of managing steroid-resistant aGVHD. Antilymphocyte globulin may reduce the severity but has not improved overall survival. Alemtuzumab (Campath; anti-CD52+ monoclonal antibody) may be effective in some cases but carries a high risk of infection as it depletes both T and B cells. Anti-interleukin-2 receptor antibodies (daclizumab, basiliximab) may reduce aGVHD but most patients go on to develop severe cGVHD. Anti-TNFα agents (etanercept, infliximab) may also produce responses in some cases but infections and failure in grade III to IV aGVHD limit usefulness. For treatment of cGVHD, pred- nisolone is usually started at a lower dose, 1 mg/kg body weight, but may need to be continued for months or years. Some steroid- sparing effect may be achieved by adding ciclosporin but this has no significant overall effect on morbidity or mortality. Rituximab (monoclonal chimeric anti-CD20 antibody) produces mainly par- tial responses in most patients with skin cGVHD or musculoskel- etal problems and imatinib may reduce fibrosis. Extracorporeal photopheresis has been used with success in patients with lung, gut, and sclerodermatous cGVHD, although its cost and availability is Table 22.8.2.3 Clinical staging of acute GVHD Clinical stage Organ involvementa Skin Liver Gut + Maculopapular rash <25% of body surface Bilirubin 30–50 µmol/litre (2–3 mg/dl) Diarrhoea 0.5–1 litre/day and/or persistent nausea ++ Maculopapular rash 25–50% of body surface Bilirubin 50–100 µmol/litre (3–6 mg/dl) Diarrhoea 1–1.5 litre/day +++ Generalized erythroderma Bilirubin 100–250 µmol/litre (6–15 mg/dl) Diarrhoea >1.5 litre/day ++++ Desquamation and bullae Bilirubin > 250 µmol/litre (>15 mg/dl) Pain ± ileus Clinical grade Stage Skin Liver Gut Functional impairment 0 (none) 0 0 0 0 I (mild)

- to 2+ 0 0 0 II (moderate)
- to 3+
-

III (severe) 2+ to 3+ 2+ to 3+ 2+ to 3+ 2+ IV (life-threatening) 2+ to 4+ 2+ to 4+ 2+ to 4+ 3+ a Confirmation may require biopsy.

section 22 Haematological disorders 5586 limiting. There is a need for randomized controlled trials to properly assess these second-line treatments to identify the most effective. Immune reconstitution and infection in HSCT The immunosuppression of the conditioning, the cytotoxicity of the agents used, and above all the severe depression of the immune response caused by GVHD, both acute and chronic, all contribute to the prolonged deficiency of cellular and humoral immunity and hence the high risk of infection in the post-transplantation period (Fig. 22.8.2.2). The duration may be exacerbated by prior chemo- therapy, T-cell depletion, and subsequent immunosuppressive treatment of GVHD. Agranulocytosis develops as soon as the condi- tioning regimen is started and continues until the graft is established and neutrophils return, usually after 2 to 4 weeks. During this phase, bacterial and fungal infections are common. Patients are managed in isolation facilities, preferably with laminar airflow to remove environmental pathogens, particularly aspergillus. Prophylactic antimicrobials including antifungals (e.g. fluconazole 100–400 mg/ day), and antivirals such as aciclovir 200 to 400 mg four times a day to prevent herpes simplex reactivation are used to cover the neutro- penic and lymphopenic phase. Antibacterials are occasionally used prophylactically. The use of ciprofloxacin and other broad-spectrum antibiotics increases the risk of Clostridium difficile infection and pseudomembranous colitis. Gram-positive infection, particularly by Staphylococcus epidermidis, is common because of the use of indwelling catheters. Pneumocystis jirovecii (previously carinii) is a major hazard, especially when steroid treatment is given for GVHD and/or CD4+ T-cell count is low. Co-trimoxazole 480 mg twice daily three times a week should be given until the risk of cGVHD has passed. CMV pneumonitis following CMV reactivation was a major cause of transplant-related mortality before the introduc- tion of effective antiviral agents such as ganciclovir. Ganciclovir is myelosuppressive so its use following early evidence of reactivation rather than automatic prophylaxis is preferred when detection of CMV by PCR or CMV antigenaemia are available. Newer antiviral agents continue to reduce the risk of CMV disease after reactiva- tion, together with novel cellular therapies including the adoptive transfer of CMV-specific T cells. Patients with cGVHD have im- mune deficiency similar to splenectomized patients and should re- ceive penicillin V 250 mg twice a day (or erythromycin if allergic to penicillin) for lifelong prophylaxis against encapsulated organisms, Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitides. A revaccination programme should be instituted once cellular and humoral immunity are reconstituted, usually 1 to 2 years after the transplant. Blood transfusions The intense immunosuppression of conditioning produces a risk of engraftment by stem cells present in transfusion products. Where transfusion is necessary, the products must be irradiated to prevent proliferation of cells. Long-term follow-up Life expectancy of recipients alive 2 years after transplant is reduced compared to matched controls, main causes of late death being relapse, cGVHD, infection, and increased risk of solid tumours. GVHD also increases the risk of later cardiovascular-related events (hypertension, diabetes, and dyslipidaemia). Immune suppression may be stopped once the graft is fully established and tolerance complete (usually 6 months to 2 years after transplant) but lifelong follow-up is still required. Management of relapse Patients transplanted for leukaemia, particularly chronic myeloid leukaemia, who develop aGVHD and/or cGVHD have less relapse, though not better survival, than

patients without GVHD. Relapse may be effectively managed by giving donor lymphocyte infusions though this increases the risk of cGVHD. There is a hierarchy of the GVL effect: chronic myeloid leukaemia being the most respon- sive to GVL, some effect in acute myeloid leukaemia, less in acute lymphoblastic leukaemia, and variable in lymphomas and myeloma. Indolent lymphoma and Hodgkin lymphoma are more responsive Viral Fungal Bacterial Gram-negative Gram-positive HSV Candida Aspergillus Encapsulated Days 0 50 100 150 Infections Risk factors Cellular and humoral immune deficiency VZV/Late CMV Adeno/RSV/CMV Central lines Acute GVHD Neutropenia Chronic GVHD Fig. 22.8.2.2 Risk factors and timing of high-risk infections following HSCT.

 to GVL. Donor lymphocyte infusions now form part of the man- agement plan after transplantation for relapse in reduced-intensity transplantations when T-cell depletion is used.

Indications for haemopoietic stem cell transplantation

The main indications are shown in Table 22.8.2.1. They fall broadly into two groups. In the first, donor stem cells are used for replace- ment therapy—a rather crude form of gene therapy—for inherited disorders. In the second group, donor stem cells are used in ma- lignant disease as an adjunct to chemotherapy, both by providing rescue from intensive cytotoxic chemo/radiotherapy and through the GVL effect. It is in this group that uncertainties remain as to the most appropriate timing as well as effectiveness of allogeneic trans- plantation. Randomized controlled trials have proved difficult to complete and much of the evidence is based on registry data, single-centre studies, or historical controls. At the same time that the results of HSCT have improved, the results of chemotherapy and newer agents have also become better. Nevertheless, particu- larly in children and younger adults, allogeneic transplantation is widely used with significant success, particularly for relapsed con- ditions where conservative management offers no potential for cure. There is a very marked inverse relationship between success of transplantation and age, children having much less transplant- related morbidity and mortality due to reduction in infectious complications and GVHD. Children also tolerate a higher degree of HLA mismatching than adults. The upper age limit for allo- geneic transplant has continued to rise as results improve and the use of reduced-intensity conditioning regimens has become wide- spread, with some centres routinely transplanting patients up to the age of 65 to 70 years. However, the transplant- related mor- bidity and mortality at this age may be very marked. As would be expected, results of allogeneic transplantation are best in low-risk groups, in first complete remission or with chemosensitive disease, and are worst in relapsed and resistant disease. However, it was in this last group that the potential benefits of allogeneic transplant- ation were first clearly demonstrated by Thomas and his group in Seattle. In most protocols for the management of leukaemias, the inclusion of allogeneic transplantation, where a suitable sibling donor is available, is considered either up-front or as a form of rescue in younger patients. The results of fully matched unrelated donor transplantations continue to improve and are now closer to those achieved with matched sibling donors. Recent advances in developing tailored conditioning regimens and GVHD prophy- laxis for haploidentical transplants has seen an increase in the use of HLA antigen-mismatched stem cells for patients where no other options are available.

Indications for autologous transplantation

The use of autologous HSCs for treatment of malignant disease can only be considered a form of rescue from increased chemotherapy since there is no GVL effect. Where there may be tumour antigens that are amenable to immune therapy, attempts have been made to induce specific immunotoxicity, so far without clear-cut benefit. On the other hand, autologous stem cell rescue does allow greatly in- creased intensity chemotherapy regimens for lymphoma, myeloma, and a variety of solid tumours with shortening of hospital stay— indeed, in

some cases treatment can be managed in an outpatient setting—and a prolonged course of therapy with repeated rescue from stored cells. Autologous stem cells will also provide the vehicle for gene therapy once techniques for gene insertion and long-term expression become practical. Cellular therapies Over the last 10 years, the development of antigen-specific T-cell therapies has moved from research laboratories into the clinic and there is now increasing evidence from early-phase clinical trials for their efficacy in treating chemotherapy-resistant haematological malignancies. Autologous tumour antigen-specific T cells can be isolated from tumour biopsies and expanded ex vivo (tumour infil- trating lymphocytes) or stimulated ex vivo in the presence of the relevant tumour antigen (tumour reactive autologous T cells) prior to reinfusion into the patient following lymphodepleting condi- tioning (to enhance homeostatic expansion of the infused T cells). As the reinfused T cells are autologous there is no risk of rejection, but their persistence and in vivo antitumour effects are determined by their fitness and ability to survive in the nutrient-poor and im- munosuppressive tumour microenvironment. They may also be subject to immunological tolerance mechanisms. More promising are gene-modified immune cells, typically CD8+ and/or CD4+ T cells which have been genetically engineered to express antigen receptors—either a physiological T cell receptor (TCR) or a syn- thetic chimeric antigen receptor (CAR), which is based on an anti- body fragment and contains an engineered intracellular signalling domain. The introduced receptors confer antigen specificity and as such redirect patient's own T cells to MHC-presented peptide (rec- ognized by TCRs) or cell surface antigens (recognized by CARs) on the target malignant cells. Clinical-grade retroviral and/or lentiviral vectors are used to introduce the antigen receptors and redirect spe- cificity. Similar technology is now being used to enhance immune cell function further by altering homing characteristics, differenti- ation status, and susceptibility to immune suppressive agents. Future directions for haemopoietic stem cell transplantation • Better understanding and treatment of GVHD, particularly cGVHD. • Wider use of alternative donors, such as UCB donations and haploidentical donors. • Further clinical trials to establish best practice, conditioning regimen, and indications. • Harnessing the GVL effect and preventing exhaustion of donor- derived T cells. • Development of cellular therapies after transplantation including gene-modified immune cells. • Continued exploration of genetic modification of HSCs for treat- ment of inherited disorders.

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