Transplantation of hematopoietic cell grafts containing pluripotent hematopoietic stem cells (HSCs) after myeloablative or nonmyeloablative conditioning regimens reconstitutes immuno-hematopoiesis. Sites from which HSC can be harvested and then used for transplantation include bone marrow, peripheral blood, and the umbilical cord. Patients may serve as their own donors (autologous) or may receive HSC from other individuals (related or unrelated). Hematopoietic cell transplantation (HCT) is done for a variety of therapeutic indications: (1) to support hematopoiesis after myeloablative doses of total body irradiation (TBI) and chemotherapy, (2) to establish a graft-versus-leukemia or tumor (GVL or GVT) reaction, or (3) to replace diseased tissues of hematologic or immunologic origins. The advances in supportive care after transplantation have resulted in improved outcomes, and HCT has become more accepted as a therapeutic modality that can be successful in otherwise life-threatening diseases. In the 2003–2007 period as compared with the 1993–1997 period, a 60% reduction in day 200 nonrelapse mortality and a 41% reduction in overall mortality was observed.1 There were also significant reductions in severe graft-versus-host disease (GVHD), opportunistic infections, and organ damage.

The focus of this chapter is on the general indications for HCT, sources of stem cells, conditioning regimens, transplant biology issues, and complications of HCT; other chapters provide detailed discussions of the indications and results of HCT in relation to specific diseases (Table 102.1).

HISTORICAL PERSPECTIVE

Early Preclinical Studies

After the effects of radiation on hematopoiesis became evident during World War II, Jacobson and colleagues reported that mice survived an otherwise lethal exposure to TBI if the spleen was shielded (Fig. 102.1).2 Radiation protection was also conferred by infusion of bone marrow.3 A running syndrome developed after recovery of hematopoiesis when the infused marrow was from a donor of a different mouse strain.4 This syndrome was due to GVHD, a complication that was soon recognized to limit the use of allogeneic marrow transplantation in humans. In further studies in mice, methotrexate and 6-mercaptopurine were found to be effective in inducing immune tolerance or ameliorating the graft-versus-host (GVH) reaction.5

The dog served as a random-bred large animal model for studies of principles and techniques of bone marrow transplantation applicable to humans. The dog was the first random-bred species in which it was demonstrated that the results of in vitro histocompatibility typing could predict the outcome of marrow transplantation.6 Littermates genotypically identical for the major histocompatibility complex (MHC) survived longer after marrow transplantation than did their MHC-nonidentical siblings. However, despite the MHC genotypic identity, GVHD was still potentially severe in many but not all dogs. This indicated that other factors identified as minor histocompatibility antigens (mHc) were involved in the development of GVHD.

Pharmacologic immunosuppression with a calcineurin inhibitor

| TABLE 102.1 |
| DISORDERS TREATED BY TRANSPLANTATION |
| Nonmalignant |
| Aplastic anemia |
| Fanconi’s anemia |
| Diamond-Blackfan syndrome |
| Sickle cell disease |
| Thalassemia |
| Paroxysmal nocturnal hemoglobinuria |
| Myelofibrosis |
| Congenital neutropenia |
| Chediak-Higashi syndrome |
| Chronic granulomatous disease |
| Glanzmann’s thrombasthenia |
| Osteopetrosis |
| Gaucher’s disease |
| Mucopolysaccharidosis |
| Mucolipidoses |
| Immune deficiencies |
| Malignant |
| Acute nonlymphoblastic leukemia |
| Acute lymphoblastic leukemia |
| Hairy cell leukemia |
| Myelodysplasia |
| Chronic myelogenous leukemia |
| Chronic lymphocytic leukemia |
| Hodgkin’s disease |
| Non-Hodgkin’s lymphoma |
| Multiple myeloma |
| Solid tumors |
(i.e., cyclosporine or tacrolimus) or methotrexate for prevention of GVHD improved survival after allogeneic marrow grafting.\textsuperscript{7,8} It was then established that methotrexate and cyclosporine in combination were more effective than either used alone.\textsuperscript{9} The efficacy of a calcineurin inhibitor and methotrexate combined for GVHD prevention was subsequently confirmed in clinical trials and remains the standard in many transplant programs.

**Early Clinical Studies**

Bone marrow was the first commonly used source of HSC for transplantation. Bone marrow transplantation from human leukocyte antigen (HLA)-identical sibling donors was first successfully used by two groups in 1968 to treat patients with immunologic deficiencies.\textsuperscript{10,11} After extensive preclinical studies of GVHD, Thomas et al. then reported the successful transplantation of marrow from a HLA-identical sibling donor for aplastic anemia in 1972.\textsuperscript{12} Five years later, the same group reported their experience in 100 patients with end-stage leukemia treated with allogeneic marrow transplantation.\textsuperscript{13} Allogeneic HCT from an HLA-matched related or unrelated donor is now considered standard therapy for many malignant and nonmalignant hematologic diseases.

**HEMATOPOIETIC STEM CELLS**

The HSC is defined as a cell with the ability to achieve long-term reconstitution of both myeloid and lymphoid lineages. To fulfill these criteria, HSC must be able to self-renew and be pluripotent. In vitro colony-forming units (e.g., CFU-GM, CFU-Meg, CFU-E, BFU-E) are progenitor cells that cannot reconstitute hematopoiesis, whereas populations of cells enriched for long-term culture-initiating cells can, at least in the mouse, rescue lethally irradiated recipients.\textsuperscript{14,15} No accepted in vitro assays for human HSC are available currently. A population of small mononuclear cells enriched for HSC can be identified by (1) the presence of the CD34 and CD133 antigen, the absence of lineage-specific antigens, and high content of aldehyde dehydrogenase and (2) the exclusion of fluorescent vital dyes including Rhodamine\textsuperscript{123} and Hoechst 33342 (Table 102.2).\textsuperscript{16–19,20} In humans and other species, successful sustained engraftment was achieved with isolated CD34-positive cells confirming that a true “stem cell” is contained within this population.\textsuperscript{21,22–24}

The true extent of the pluripotency of adult marrow-derived stem cells remains under investigation. Several studies have reported that populations of HSCs may contribute to regeneration of muscle, osteoblasts, hepatocytes, and neuronal and nonneuronal cell types of the brain.\textsuperscript{25–27} However, some of the

**TABLE 102.2**

<table>
<thead>
<tr>
<th>CHARACTERISTICS OF HUMAN HEMATOPOIETIC STEM CELL (HSC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Cell Surface Antigen Expression</strong></td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>*CD34-positive</td>
</tr>
<tr>
<td>CD133-positive</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase</td>
</tr>
<tr>
<td>Low Positive</td>
</tr>
<tr>
<td>Thy 1 (CDw90), c-KIT</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>CD38, CD33, T and B cell markers, CD71, DR</td>
</tr>
<tr>
<td><strong>B. Dye Exclusion</strong></td>
</tr>
<tr>
<td>Rhodamine\textsuperscript{123}, Hoechst 33 342</td>
</tr>
</tbody>
</table>

*CD34 negative HSC have been identified in mice.
experimental observations may have resulted from cell fusion, technical artifact, or culture induced changes in cellular gene expression. Nevertheless, there is agreement that if “plasticity” of circulating HSCs occurs, it is likely to be a rare event.28

**Identification of Cell Populations Enriched for Hematopoietic Stem Cells**

The CD34 antigen is a cell surface type I transmembrane protein which is highly O-glycosylated and expressed primarily on hematopoietic progenitor cells and vascular endothelium from many tissues.29,30,31 It is also expressed on stromal cell precursors identified by the STRO-1 antibody. Cell surface expression of CD34 is developmentally regulated in hematopoiesis and is inversely related to the stage of differentiation such that CD34 expression is absent beyond the committed progenitor stage. The functional significance of CD34 expression on hematopoietic progenitor cells, stromal cells, and developing blood vessels is unknown, except that CD34 on vascular endothelial cells binds to L-selectin.32 The CD34 antigen is expressed on 1% to 5% of normal human adult marrow cells, up to 1% of mobilized peripheral blood cells, and by 2% to 10% of normal fetal liver and marrow cells.29,33 Approximately 90% to 95% of the CD34-positive cells express antigens indicating commitment to the lymphoid or myeloid lineages.34,35 Purified populations of HSC can be obtained with strategies that lineage-deplete a CD34-positive population of cells using monoclonal antibodies specific for DR, CD33, CD38, CD71, and B and T cell markers (Table 102.2). Other work suggests that human HSC are Thy-1low, c-KITlow, Rhodamine123low and CD133-positive.36–41 In vivo preclinical models for studying populations of purified human HSCs based on the aforementioned characteristics include transplantation into SCID-Hu mice or into fetal sheep.40,42,43

Enriched populations of autologous CD34-positive marrow cells or blood cells, in both animal and human studies, have been shown to protect from myeloablative doses of radiation or chemotherapy.22,44,45 Conversely, the CD34-negative subset of the marrow was not protective.46,47 Complete and stable donor hematopoietic chimerism has also been shown in humans after transplantation with allogeneic CD34-enriched cells from the peripheral blood.24,48 Thus, CD34-positive hematopoietic cells are capable of long-term stable reconstitution of multiple hematopoietic lineages.

**Sites of Hematopoiesis and for Collection of Hematopoietic Stem Cells**

In the developing human embryo, the production of hematopoietic cells shifts to the liver from the yolk sac after 6 weeks of gestation. At 16 weeks of gestation, the most active site of hematopoiesis is the fetal liver. At the end of gestation, essentially all hematopoietic production derives from the marrow with only small contributions from the liver and spleen. Umbilical cord blood is enriched for HSC. The number of progenitors in one cord blood unit was comparable to the number of progenitors from adult marrow that had been reported to achieve successful engraftment.49 In early clinical studies, transplantation of autologous peripheral blood HSC resulted in reconstitution of hematopoiesis following myeloablative chemotherapy or chemoradiotherapy; however, obtaining sufficient HSC required a prolonged period of collection.50 To overcome this problem, granulocyte-colony-stimulating factor (G-CSF) can be administered to mobilize HSC from the marrow to the peripheral blood. Collections from G-CSF-mobilized peripheral blood yielded similar or greater numbers of HSC than that harvested from marrow.54 Combining chemotherapy and G-CSF administration for mobilization resulted in higher yield of CD34-positive cells than G-CSF alone.55 Plerixafor is a small molecule that reversibly inhibits chemokine stromal cell-derived factor-1a binding to its cognate receptor CXC chemokine receptor 4. In a randomized clinical trial, G-CSF with plerixafor was more effective for collection of stem cells patients with myeloma.56 In this study, a total of 54% of plerixafor-treated patients collected a target number of 6.0 x 106 CD34+ cells/kg after one apheresis, whereas 56% of placebo-treated patients required 4 daily aphereses to achieve this target. Other cytokines have been demonstrated to mobilize HSC into peripheral blood, including stem cell factor, granulocyte/macrophage-CSF, interferon-γ, IL-6, IL-8, and IL-3 ligand.57,58 While most normal donors receive only G-CSF for mobilization of HSC for allogeneic transplant, mobilization strategies including plerixafor or chemotherapy are now routinely used for the collection of autologous HSC from the peripheral blood of patients with hematologic malignancy.59,60,61 Plerixafor is not indicated for use in patients with acute leukemia.

Monoclonal antibodies specific to the adhesion molecule VLA-4 (very late antigen-4 or α4β1 integrin) and VCAM-1 (vascular cell adhesion molecule-1) can also mobilize hematopoietic progenitors in nonhuman primates.62,63 An essential step contributing to the release of hematopoietic progenitors from the marrow may be the cleavage of VCAM-1 expressed on stromal cells by neutrophil proteases following the administration of G-CSF.64 Hematopoietic progenitors reversibly downregulate VLA-4 expression and adhere significantly less to stroma and fibronectin during mobilization.65 VLA-4 integrin expression is restored after progenitors are removed from the in vivo mobilizing milieu, which may account for their homing properties after transplantation.

**SeleCtion of stem cell source**

Autologous, syngeneic, and allogeneic are the three general categories of HSC grafts (Table 102.3). In general, the origin of HSC used for HCT is based on both the availability of the donor and the type of disease for which the patient is being transplanted. While autologous HSC should be available for most patients, extensive prior cytotoxic therapy or heavy involvement of marrow with malignant cells may preclude the use of this source of HSC. Although the preferred allogeneic donor is an HLA-identical sibling, fewer than 30% of patients have access to this source. Availability of HLA-identical sibling donors for pediatric patients may be less than this because of the smaller family sizes now compared to previous generations. Syngeneic donors are available in less than 1% of cases, and phenotypically HLA-matched or one-antigen-mismatched haploidentical family donors are available in less than 5% of cases.66 Approximately 30% to 40% of patients may identify a phenotypically HLA-matched unrelated donor from the volunteer registries.67 The availability of unrelated (or related) umbilical cord blood banks increases the chances of successfully identifying a compatible allogeneic graft source for both pediatric and adult patients.68–70 HLA-haploidentical donors are also available for the majority of patients.

The disease for which transplantation is being considered is another important determinant for choice of stem cell source. Autologous, syngeneic, or allogeneic HSC can support hematopoietic recovery after myeloablative chemoradiotherapy for malignant hematologic and nonhematologic diseases. For acquired disorders of marrow function (e.g., aplastic anemia), syngeneic or allogeneic HSC are required.71 Patients with congenital hematopoietic or immunologic defects (e.g., thalassemia, severe combined immunodeficiency syndrome) require transplantation with both autologous stem cells and gene-modified autologous stem cells.72–75
Autologous Source of Stem Cells

Autologous marrow or mobilized peripheral blood stem cells are the grafts of choice for many patients. Autologous stem cell support is most commonly derived from “mobilized” peripheral blood stem cells instead of marrow because of faster hematopoietic recovery in the recipient. Peripheral blood stem cell transplants have decreased the time required for hospitalization. Autologous stem cell support after myeloablative therapy has been successful for treatment of acute myelogenous leukemia (AML), non-Hodgkin lymphoma, and Hodgkin lymphoma. Disease-free survival was prolonged in patients with multiple myeloma receiving a single autologous or two sequential autologous transplants. Significant transplant-related complications encountered most frequently include infections and organ toxicity of the liver or lung, caused in large part by the high-dose myeloablative cytotoxic therapy. A GVHD-like syndrome (also known as the engraftment syndrome or pseudo-GVHD) of the skin and gastrointestinal tract has been described, but it is generally not frequent or severe.

Allogeneic Source of Stem Cells

Transplantation from Related Donors

Hematopoietic cell grafts from related donors may be HLA-identical or HLA-haploidentical. The preferred allogeneic donor has been a genotypically HLA-identical sibling. A genetic match at the HLA loci between siblings is confirmed by the genotyping of 5 HLA loci including HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1. If an HLA-identical sibling is available, patients with hematologic malignancies should be transplanted with peripheral blood stem cells rather than marrow. Two phase 3 studies have now shown an improved disease-free and overall survival with transplantation of peripheral blood stem cells (Fig. 102.2). Ten-year follow-up of the study by Bensinger et al. showed that the benefit persisted for disease-free survival, but the likelihood of overall survival was not significantly different between the 2 groups. The 10-year cumulative incidence of chronic GVHD and the duration of systemic immunosuppression were similar between the 2 groups. A third study concluded that peripheral blood was an equivalent source of HSCs compared with marrow if administered to patients with standard-risk leukemia, since a significant difference in survival could not be demonstrated. All 3 studies showed an accelerated recovery of neutrophil counts in the group receiving peripheral blood stem cells. Although most studies have not shown a significant increase in the incidence of acute GVHD in the peripheral blood stem cell group, the incidence of chronic GVHD was significantly greater. Higher doses of CD34-positive cells in the peripheral blood stem cell graft (> 8.0 × 10^6/kg) have been significantly associated with the development of chronic GVHD. Since outcome is unlikely to be improved if there is an increased risk of chronic GVHD from the use of peripheral blood stem cells, patients with nonmalignant disorders are still being transplanted with marrow in some centers. With a median follow-up of 6 years, survival of aplastic anemia patients transplanted with marrow was 88% (Fig. 102.3). In a retrospective analysis by CIBMTR, rates of chronic GVHD and overall mortality were greater after transplantation with peripheral blood stem cells than marrow, especially in the younger patient population (<20 years of age). Similar survivals to those observed after HCT for aplastic anemia have been reported in patients transplanted with marrow for hemoglobinopathies.
Outcomes have improved after HCT from HLA-matched unrelated donors because of improved supportive care and high-resolution HLA typing. Outcomes after HCT from an unrelated donor are now comparable to what is observed after HCT from an HLA-identical sibling. In a study of 2,223 adult AML patients, the overall survival after transplantation from 8/8 HLA-matched donors was similar to that observed after transplantation from an HLA-identical sibling \( (RR = 1.03; P = 0.62) \). The risk of acute GVHD, however, was lower after transplantation from an HLA-identical sibling.

The relative importance of the various HLA loci has been defined for HCT from unrelated donors. In a study from CIBMTR of 3,857 transplants performed from 1988 to 2003, it was observed that high-resolution DNA matching for HLA-A, HLA-B, HLA-C, or HLA-DRB1 (8/8 match) was the minimal level of matching associated with the highest survival. A single mismatch at HLA-A, HLA-B, HLA-C, or HLA-DRB1 (7/8 match) was associated with an increased mortality compared to an 8/8 match. A single mismatch at HLA-B and HLA-C appeared better tolerated than a mismatch at HLA-A and HLA-DRB1. A mismatch at HLA-DQ or HLA-DP loci did not have an effect on survival. Ninety-four percent of the transplants in this study were performed with marrow grafts. In a follow-up study, the association between HLA matching and outcomes was investigated for transplantation of peripheral blood stem cells from unrelated donors. Survival was better with 8/8 HLA matching compared to 7/8 HLA matching. Single HLA-C antigen mismatches were associated with an increased risk of treatment-related mortality and grade III-IV acute GVHD. HLA-B antigen/allele mismatch was associated with an increased risk of grade III-IV acute GVHD with no effect on survival. No significant differences in outcomes were observed with HLA-C allele mismatches, HLA-A antigen/allele mismatches, or HLA-B1 mismatches compared to 8/8 HLA-matched pairs.

The differences in the reported associations for HLA mismatches between marrow and peripheral blood stem cell grafts may result from differences in cell numbers in the graft as well as the graft composition.

Disease status is an important consideration in the search process. In patients with advanced malignancies, survival after transplantation from an HLA-matched or 1-antigen/allele mismatched HLA-mismatched unrelated donor was similar. Therefore, when transplants cannot be delayed because of disease status, selection of a donor with the fewest HLA mismatches may be an alternative choice for patients without a completely HLA-matched donor.

Because of the possible higher risk of acute GVHD, studies of peripheral blood stem cell transplantation from unrelated donors started later than those done from HLA-identical donors. A recently completed randomized clinical trial (BMTCTN 0201) comparing marrow to peripheral blood stem cells observed that there was no difference in overall survival, disease-free survival, nonrelapse mortality, relapse, or acute GVHD outcomes at 2 years between the 2 arms. There was a significantly increased risk of chronic GVHD.

### Transplantation with Umbilical Cord Blood Grafts

There are several advantages to the use of umbilical cord blood when compared to unrelated donor peripheral stem cell or marrow product harvested from adults. First, umbilical cord blood represents a potentially nonlimiting donor source for transplantation. At present, over 550,000 HLA typed cord bloods are banked and are conceivably available with several days notice (www.bmdw.org). Many of the banked specimens are from underrepresented ethnic and racial groups, thus expanding the potential donor pool for individuals poorly served by the unrelated donor marrow registries. The total nucleated cell dose required for successful engraftment is 10-fold less than that required for conventional peripheral blood or marrow transplant. The transmission...
and grade III-IV GVHD, was 81% and 23%, respectively. Adults) after transplantation with umbilical cord blood grafts in adults were comparable to those reported in children.

Concern about the low cell count for the larger body size, outcomes from unrelated donors (relative to the umbilical cord blood cells from unrelated donors (99 were from umbilical cord blood. These results were later confirmed by another group. Initially, recipients of umbilical cord blood cell transplants had an increased number of HLA mismatches but a lower risk of acute and chronic GVHD compared to recipients of unmanipulated marrow from unrelated donors. The day 100 mortality was higher, however, in the cord blood group, possibly because of the significantly delayed recovery of hematopoiesis and immunity after transplantation. These results were later confirmed by another group. It was concluded that the use of umbilical cord blood was an option for children with acute leukemia lacking an acceptably matched unrelated marrow donor. In children who had received umbilical cord blood or bone marrow grafts from HLA-identical siblings, the umbilical cord blood group had a lower risk of acute and chronic GVHD (relative risk 0.41 [P = 0.001] and 0.35 [P = 0.02]), respectively. Survival was similar in both groups. The progenitor cell and CD34-positive cell content of the umbilical cord blood graft predicted the rate of neutrophil recovery after transplantation. The reported low incidence of severe GVHD after transplantation of umbilical cord blood cells from unrelated donors (relative to the degree of HLA disparity) may be related to the decreased immunocompetence of the fetal blood cells compared to adult cells. Two years after transplantation, T cell receptor rearrangement excision circles (TRECs), a measure of recent thymic output, were greater in recipients of umbilical cord blood than in recipients of marrow grafts, suggesting complete immune recovery despite the low number of cells infused.

Initially, recipients of umbilical cord blood cell transplants had been mostly children. Even though, at first, there was a concern about the low cell count for the larger body size, outcomes in adults were comparable to those reported in children. Prospective trials comparing cord blood transplantation to unrelated peripheral blood/marrow transplants are not available; however, two large registry or retrospective studies summarizing consensus exists for acute or chronic leukemias have been published. Slower engraftment kinetics, reduced engraftment rates, and decreased acute and chronic GVHD rates and severity were observed in the cord blood transplantation groups. Rates of relapse reported for the two studies were similar between the 2 groups. Transplant-related complications, including delayed recovery of blood counts after cord blood transplantation, may be reduced in the adult population with reduced intensity conditioning. Transplantation of multiple cord blood units was investigated to determine if recovery of neutrophil and platelet counts after transplantation could be improved. After the infusion of two umbilical cord blood grafts into adult patients, the median day to neutrophil recovery was 24 (range 12 to 28) in 23 adult patients compared to 27 (range 13 to 59) in another study of 68 adult patients transplanted with a single cord blood unit. This observation of improved recovery times was not confirmed in a retrospective study at a single institution. After transplantation with 2 cord blood units, only one unit eventually dominates and engrafts long-term. Higher CD3+ cell dose and percentage of CD34+ viability were associated with unit dominance. Higher dominant unit CD34+ cell dose and colony-forming unit doses were associated with higher sustained engraftment and faster neutrophil recovery. Mechanistically, the dominant cord mounts an allogeneic immune response mediated by CD8+ T cells against the non-dominant unit.

Outcomes after double cord blood transplantation were compared with outcomes after transplantation from HLA-matched related donors, HLA-matched unrelated donors, and 1-antigen HLA-mismatched unrelated donors. Leukemia-free survival at 5 years was similar for all donor types. The risk of relapse was decreased in the double cord blood transplantation group compared to the other donor types, but the risk of nonrelapse mortality was increased. The lower risk of relapse after transplantation from a double cord blood unit compared to a single cord blood unit had been previously seen in another retrospective study, although a significantly increased risk of acute GVHD was also observed. Sharing of one or more HLA-A, B, or DRB1 loci between the inherited paternal MHC alleles (IPA) of the donor and the recipient after unrelated cord blood transplantation leads to superior leukemia-free survival with no increase in acute GVHD, a result attributed to maternally derived anti-IPA elements persisting in the cord blood. Umbilical cord blood grafts should be considered for both pediatric and adult patients lacking a suitably matched unrelated donor or unable to wait for an unrelated search to be completed.

**COLLECTION OF HEMATOPOIETIC STEM CELLS FOR TRANSPLANTATION**

**Marrow**

Marrow is obtained by multiple aspirations from the posterior iliac crest under general or epidural anesthesia. The anterior iliac crest (or the sternum) may also be harvested if larger quantities of marrow are required. The target volume of marrow for transplant is 10 to 15 ml/kg of recipient or donor weight, whichever is the smaller individual. Marrow is collected with heparinized syringes through large bore needles and placed into small amounts of culture medium. The collected marrow must be filtered prior to intravenous transfusion into the recipient to remove small particles or clots. If the patient and donor are ABO-incompatible and there are high anti-A or -B titers, the marrow can be red blood cell-depleted or plasma-depleted, depending on whether it is a major or minor ABO mismatch. In some cases of a major ABO mismatch, plasmapheresis of the recipient may be effective in reducing the high anti-A or anti-B titers so that RBC depletion of marrow is not required. Marrow is infused immediately after harvesting, but delays of 24 hours may occur without adverse consequences. Such delays are common when marrow is shipped to a transplant program after harvest from an unrelated donor. In an analysis of marrow harvest characteristics of 1,549 donors, the median total nucleated cell count from the marrow was 2.5 × 10^9/kg recipient weight. Life-threatening complications from marrow harvesting, usually related to the administration of anesthesia, were reported in 0.27% to 0.4% of the donors.

**Peripheral Blood**

HSC circulate in the peripheral blood but the concentration is very low and it requires multiple aphereses to collect adequate
numbers. The number of aphereses may be reduced to one or two sessions when HSC are mobilized to the peripheral blood after the administration of cytokines alone or in combination with chemotherapy or plerixafor. An effective mobilization strategy of autologous HSC for patients with malignancy is chemotherapy in conjunction with G-CSF, 6 μg/kg/day. After chemotherapy, patients are apheresed when the total white blood cell count has recovered to 1,000/μl or the CD34-positive cell count in the peripheral blood is at least >10/μl. For patients not requiring chemotherapy or normal allogeneic donors, mobilization is with G-CSF alone (5 to 16 μg/kg) by daily subcutaneous injections for 5 to 8 days. These doses of G-CSF are generally well tolerated, with common side effects of bone pains, myalgias, and flu-like symptoms that are managed with acetaminophen or low-dose narcotics. Plerixafor in combination with G-CSF was effective for increasing the yields of circulating CD34+ progenitor cells and is indicated for patients with lymphoma or multiple myeloma who are being collected for autologous HCT. The recommended dose is 0.24 mg/kg/day administered subcutaneously 11 hours before the apheresis procedure. The maximum dose is 40 mg/day. Common side effects included diarrhea, nausea, fatigue, headaches, and arthralgias. Plerixafor was performed as early as day 4 after the start of G-CSF using a continuous blood flow separation technique. Apheresed products may be cryopreserved in 5% dimethylsulfoxide (DMSO) for use after thawing on the day of transplant. A more rapid sustained hematopoietic recovery of both neutrophil and platelet counts occurs with increasing numbers of CD34+ cells in the hematopoietic cell graft (up to 5 × 10^6/kg). Some investigators consider 2.5 × 10^6/kg of recipient weight a minimum dose of CD34+ cells from the peripheral blood to achieve complete autologous recovery. Platelet recovery is more rapid at higher CD34+ cell doses. Since the cell dose used in the autologous transplant setting yields consistent and prompt engraftment, it is also considered an appropriate target for collection of allogeneic HSC from the peripheral blood. Donors of peripheral blood avoid general anesthesia and other complications of marrow harvesting. If peripheral veins are inadequate, a large bore double lumen catheter for vascular access may be required. In a large analysis of safety from the NMDP (n = 2,408 donors), it was concluded that collection of peripheral blood stem cells was safe but that nearly all patients will experience bone pain and 1 in 4 will have headache, nausea, or citrate toxicity. Serious and unexpected toxicities were experienced by 0.6% of the donors, but complete recovery was universal.

Cord Blood

Umbilical cord blood cells are now being routinely collected and cryopreserved for storage in a cord blood bank. Directed-donor banking of cord blood for siblings in a current good tissue practices environment has also now been reported. After the separation of the placenta, umbilical cord blood cells are collected into a closed system which utilizes a sterile donor blood collection set. The placenta and umbilical cord can be suspended on a frame which is placed in a sterile donor blood collection with other chemotherapeutic agents (e.g., etoposide, Ara-C, and melphalan) in combination with TBI, CY, or both CTX and TBI. Other chemotherapeutic agents used in combination with CY but without TBI, including busulfan (BU)/CY or carmustine-cyclophosphamide-etoposide (BCV), and replacement of CY with fludarabine and used in combination with BU or melphalan. In one study, BCV was associated with a significant incidence of transplant-related complications and mortality. Most preparative regimens used for the treatment of malignant diseases have not been tested in Phase III studies, so it is generally unclear if any one regimen represents an improvement over those previously used. Two Phase III studies in allogeneic marrow transplantation have compared differing intensities of TBI are directed against the donor red blood cell antigens. A minor ABO incompatibility is when the isohemagglutinins in the donor plasma are directed against recipient red blood cell antigens. ABO incompatibility between the donor and the recipient has no significant effect on the incidence of graft rejection, GVHD, or survival, although bidirectional ABO mismatches were associated with a higher risk of grade III-IV acute GVHD. ABO incompatibilities may result in severe hemolytic episodes after transplantation. For major ABO incompatibilities, if the recipient isohemagglutinin titers in the plasma are high, an acute hemolytic event can be prevented by red cell depletion of the graft. Conversely, plasmapheresis may be effective in reducing the anti-donor isohemagglutinin in the recipient plasma. If the latter approach is chosen, the goal should be to reduce the isohemagglutinin titer to 1:16 or lower. If there is continued production of anti-donor isohemagglutinins in the recipient plasma after transplantation, delayed erythropoiesis or even pure red cell aplasia and persistent hemolysis may result. Although plasmapheresis and erythropoietin may be of some benefit, the hemolytic episode may persist for months after transplantation. If there are no contraindications, early withdrawal of immunosuppression may result in a more rapid resolution of the delayed hemolytic event, possibly because of a GVH reaction against the isohemagglutinin-producing cells of the recipient. To prevent hemolytic events related to minor ABO incompatibilities, the isohemagglutinins can be removed from the stem cell graft if the anti-recipient titers are high. The risk of hemolysis from a minor ABO mismatch appears to be increased after peripheral blood stem cell transplantation, possibly related to the higher content of lymphoid cells (B cells) in the graft. The development of severe hemolysis may be prevented with the administration of methotrexate after transplantation.

**CONDITIONING REGIMENS**

**Myeloablative**

Myeloablative conditioning regimens are sufficiently intense that recovery of hematopoiesis would not be expected without the support of transplanted hematopoietic progenitor cells. The ideal myeloablative conditioning regimen should fulfill the following criteria: (1) eliminate or reduce the tumor load; (2) suppress the host immune system to prevent graft rejection (not applicable to hematopoietic support with autologous cells); and (3) have tolerable nonhematopoietic toxicity. The first conditioning regimens consisted of TBI, alone or in combination with cyclophosphamide (CY). TBI is an effective antineoplastic modality that is both cycle nonspecific and immunosuppressive. CY is a chemotherapeutic agent with immunosuppressive properties that has few nonhematopoietic toxicities that are similar to TBI, and therefore can be used in combination. Other conditioning regimens which have since been developed include: (1) the replacement of CY with other chemotherapeutic agents (e.g., etoposide, Ara-C, and melphalan) in combination with TBI; (2) other chemotherapeutic agents in combination with both CY and TBI; (3) chemotherapeutic agents used in combination with CY but without TBI, including busulfan (BU)/CY or carmustine-cyclophosphamide-etoposide (BCV); and (4) replacement of CY with fludarabine and used in combination with BU or melphalan. In one study, BCV was associated with a significant incidence of transplant-related complications and mortality. Most preparative regimens used for the treatment of malignant diseases have not been tested in Phase III studies, so it is generally unclear if any one regimen represents an improvement over those previously used. Two Phase III studies in allogeneic marrow transplantation have compared differing intensities of TBI...
(1,200 cGy versus 1,575 cGy). The relapse rate was reduced in the group of patients receiving the higher dose of TBI, but was associated with an increase in complications from regimen-related toxicity and GVHD which negated any improvement in disease-free survival compared to the group receiving the lower dose of TBI. In a more recent study, conditioning with TBI 800 cGy and fludarabine was compared with TBI 1,200 cGy and CY and there was no difference in overall survival, relapse, or treatment-related mortality. The combination of BU/CY was determined to be an acceptable alternative to CY/TBI for patients with leukemia in several studies. However, in one study, patients in the BU/CY group had an increased risk of sinusoidal obstruction syndrome (SOS) of the liver and other transplant-related complications. Since this last study, it has been demonstrated that targeting of busulfan levels in the plasma may decrease the risk of relapse and severe regimen-related toxicities, contributing to an improved disease-free survival (Fig. 102.4). Monitoring levels of metabolites from cyclophosphamide may also be important to decrease the risk of liver toxicity and nonrelapse mortality.

In patients already profoundly immunosuppressed with SCID syndrome, engraftment of allogeneic stem cells from matched related siblings may occur without conditioning therapy. High-dose CY in combination with antithymocyte globulin (ATG) as a preparative regimen for patients with aplastic anemia was associated with graft rejection in less than 5% of cases. The actuarial survival rate was 88% at 6 years after transplantation (Fig. 102.3). For transplantation of patients with thalassemia or sickle cell disease, a myeloablative conditioning regimen, usually consisting of the combination of BU and CY, was thought to be necessary to prevent a high incidence of graft rejection, since most patients with these disorders had received multiple blood transfusions and were potentially sensitized to donor-derived mHC. However, reduced intensity conditioning regimens have now been shown to successfully overcome the risk of graft rejection associated with these hematologic disorders.

Although HSC engraftment may be achieved after transplantation and some patients are cured, relapse remains an important problem in patients with advanced hematologic malignancies. Since further intensification of the conditioning regimen using current modalities is unlikely to improve overall survival because of associated increases in toxicity, new strategies will be required to achieve a cure. Radiolabeled monoclonal antibodies (i.e., radioimmunonoconjugates) specific for certain lineage-specific cell surface antigens on tumor cells or sites from which the malignancy originates might enhance the efficacy of the conditioning regimen and decrease the rates of relapse.

**Nonmyeloablative**

The development of nonmyeloablative or reduced intensity regimens permitted the application of potentially curative allogeneic HCT to older patients and those patients with contraindications to intensive cytotoxic regimens. The success of the nonmyeloablative conditioning regimens in preclinical and clinical studies established new biologic principles which formed the basis of our understanding of the engraftment process after allogeneic HCT. The findings in these studies were consistent with the following conclusions about successful engraftment:

1. Myeloablative therapy is not needed, since stem cell grafts can "create their own marrow space" with a GVH effect.
2. Engraftment is promoted by posttransplant suppression of the host-versus-graft reaction.

There are multiple different nonmyeloablative conditioning regimens which have been piloted. The conditioning regimens fall into 2 broad categories: (1) reduced intensity regimens and (2) minimally myelosuppressive regimens. In general, after reduced intensity conditioning, recipients usually become aplastic from the regimen and complete chimerism is established early after transplantation. Reduced intensity conditioning regimens are of sufficiently low intensity that recovery of hematopoiesis would be expected without the support of hematopoietic progenitor cells. Although reduced in intensity, the regimen may contribute a substantial antitumor effect resulting in a tumor response early after transplantation. The minimally myelosuppressive or nonmyeloablative regimens rely on pretransplantation and posttransplantation immunosuppression to prevent graft rejection. After transplantation, a mixed chimeric state may persist for months before converting to full donor hematopoietic chimerism (Table 102.4). The regimen may be only mildly myelosuppressive resulting in a reduction in the requirements for blood products (Fig. 102.5).

### TABLE 102.4

<table>
<thead>
<tr>
<th>MEDIAN PERCENT AND CHIMERISM STATUS</th>
<th>Day 28&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Day 56&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median % (range) of donor cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T cells</td>
<td>60 (10–100)</td>
<td>91 (5–100)</td>
</tr>
<tr>
<td>PB neutrophils</td>
<td>91 (0–100)</td>
<td>99 (4–100)</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>87 (0–100)</td>
<td>95 (2–100)</td>
</tr>
<tr>
<td>Chimerism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Mixed&lt;sup&gt;d&lt;/sup&gt;</td>
<td>86</td>
<td>79</td>
</tr>
<tr>
<td>% Full Donor&lt;sup&gt;d&lt;/sup&gt;</td>
<td>14</td>
<td>21</td>
</tr>
</tbody>
</table>

<sup>a</sup> 44 evaluable patients. One additional patient–donor pair had no identifiable DNA polymorphism, and evidence of donor cell engraftment was based on red blood cell polymorphisms.

<sup>b</sup> 41 evaluable patients.

<sup>c</sup> ≤1 and ≤5% T cells of donor origin.

<sup>d</sup> ≥95% T cells of donor origin.

Early studies of nonmyeloablative transplantation focused on patients with donors who were HLA-identical siblings because the risk of graft rejection and GVHD was considered less compared to other types of donors. Risk factors for graft rejection after conditioning with TBI 200 cGy alone were no prior intensive chemotherapy and a diagnosis of CML. When fludarabine was added to low-dose TBI, the incidence of graft rejection decreased. Nonmyeloablative conditioning regimens have also been effective for transplantation from alternative sources including unrelated donors and umbilical cord blood.

The allogeneic HSC graft can induce GVL/GVT effects. The effectiveness of the GVT effect can be best observed in the responses patients have had with the minimally myelosuppressive effect. The assessment of chronic myelogenous leukemia (CML) induced by nonmyeloablative hematopoietic cell transplantation (HCT) without donor lymphoid infusion, as documented by failure of reverse transcription–polymerase chain reaction (PCR) to detect BCR-ABL transcripts. The lane described “−ve” is negative control of normal bone marrow (BM). K562 is a positive control for BCR-ABL.

In patients not otherwise eligible for a myeloablative conditioning regimen, sustained responses have been noted with AML, the chronic leukemias, lymphoma, and myeloma. A minimally myelosuppressive conditioning regimen may be appropriate for indolent hematologic malignancies including CML, chronic lymphocytic leukemia, agnogenic myeloid metaplasia, and low grade lymphomas. In those diseases in which some cytoreduction may be necessary, a reduced intensity regimen may be of more benefit. Another strategy is to use a cycle of chemotherapy or high-dose cytotoxic therapy and autologous transplantation to debulk the disease or induce a clinical remission followed by allogeneic nonmyeloablative transplantation.

The objective of this tandem approach is to consolidate the remission with a GVT reaction. The assessment of the patient, the disease type, and the disease status are required to determine the optimal approach. In retrospective comparisons...
of patient populations after myeloablative and nonmyeloablative conditioning regimens, no difference in overall survival was observed.\textsuperscript{185–187} However, relapse rates were higher in patient groups which had received a nonmyeloablative conditioning regimen.

GVHD and infections are the major reasons for transplant-related treatment failure after nonmyeloablative transplantation. Since these patients, in general, have been older and at higher risk of poor outcome based on preexisting complications at baseline, nonmyeloablative transplantation has been well tolerated compared to what could have been expected after myeloablative conditioning. The day 100 and year 1 treatment-related mortality has been reported as 3% and 16%, respectively, but significantly less than after myeloablative conditioning.\textsuperscript{188} Earlier clinical trials of nonmyeloablative conditioning regimens had given short courses of immunosuppressive therapy or early administration of donor lymphocyte infusions (DLI). GVHD tended to develop later, with many cases developing at 3 to 6 months after transplantation.\textsuperscript{189} The cumulative incidence of acute grade II–IV GVHD was less after a nonmyeloablative than a myeloablative conditioning regimen (64% versus 85%, \( p = 0.001 \)). There was no difference in the incidence of chronic GVHD. GVHD developed in many patients as late as 6 to 12 months after nonmyeloablative conditioning and HCT. The incidence of acute GVHD decreased when GVHD prophylaxis was administered for a longer period after HCT.\textsuperscript{190} Acute GVHD did not have a significant impact on the risk of progression but had an association with increased risk of nonrelapse mortality. Chronic GVHD was associated with a decreased risk of relapse or progression (\( P = 0.006 \)) and an increased probability of progression-free survival (\( P = 0.003 \)).\textsuperscript{178} Strategies for the optimal management of GVHD are required so that complications are reduced but not prevented by an effective GVT response.

In the presence of a major ABO incompatibility, delayed development of donor red cell chimerism resulting in an increased need for red blood cell products has been reported.\textsuperscript{196} Withdrawal of cyclosporine may induce a graft-mediated immune reaction against recipient isohemagglutinin-producing cells, thus resulting in a decrease in isohemagglutinin levels and improvement in donor red blood cell chimerism. The onset of CMV disease may be delayed after nonmyeloablative transplantation.\textsuperscript{191,192} Patients after nonmyeloablative conditioning with a minimally myelosuppressive regimen can generally be managed in the outpatient setting.

**ENGRAFTMENT**

Stable engraftment of a hematopoietic cell graft requires the circulation, homing, and growth of HSC. The number of CD34\(^+\) cells that are required to ensure prompt recovery of neutrophil and platelet counts is 2.5 to 5.0 \( \times 10^7 \) /kg. In addition, adequate suppression of the host immune system is required to prevent graft rejection after allogeneic HCT. To achieve adequate immunosuppression, conditioning regimens may be myeloablative or nonmyeloablative. Donor T cells are important to prevent graft rejection, even with the concomitant administration of substantial numbers of CD34\(^+\) cells.\textsuperscript{193} Although infrequently observed in humans, a single stem cell may substantially reconstitute hematopoiesis (clonal dominance) and demonstrates the considerable proliferative potential of a normal HSC.\textsuperscript{194,195} In many clinical trials of HCT, engraftment has generally been defined as the achievement of a peripheral blood neutrophil count greater than 0.5 \( \times 10^9 \) /L and evidence of donor chimerism if engraftment is being assessed after allogeneic HCT. The rate of engraftment is dependent upon the number of HSC (CD34\(^+\) cell content), purging strategies for autologous grafts, use of growth factors, and the use of methotrexate for GVHD prophylaxis.\textsuperscript{61,196,197} Graft failure may result from either an infusion of inadequate numbers of HSC, drug toxicity, infection (CMV), or graft rejection (after allogeneic transplantation). Relapse also needs to be considered in a patient with decreasing peripheral blood counts.

Graft rejection is infrequent (<1% incidence) after HCT from HLA-matched siblings and was more common after HLA-mismatched related or unrelated HLA-matched donors (2% to 6%).\textsuperscript{198} Luznik et al. reported a rejection rate of 13% after reduced intensity conditioning and HCT from an HLA-haploidentical donor.\textsuperscript{199} All but 1 patient experienced recovery of autologous hematopoiesis after rejection in this study. As grafts are rejected, peripheral blood counts may drop, and chimerism studies demonstrate the loss of donor cells in the peripheral blood and marrow. Factors that influence the risk of graft rejection include T-cell depletion of the hematopoietic cell graft, increased HLA disparity, the intensity of the conditioning regimen and posttransplant immunosuppression, pretransplant sensitization of the host against donor alloantigens, and the number of HSC in the graft.\textsuperscript{98,199–201} Previous chemotherapy has been associated with a reduced risk of graft rejection after nonmyeloablative conditioning.\textsuperscript{202} T-cell engraftment kinetics were also improved in patients with higher steady state concentrations of the active mycophenolate mofetil (MMF) metabolite (achieved with three times daily dosing instead of twice daily) without increased risk of relapse, albeit with a modest increase in the rate of CMV reactivation.\textsuperscript{203} Clinically, hematopoietic growth factors have not been demonstrated to be effective in preventing graft rejection. Historically, the occurrence of graft rejection after a myeloablative conditioning regimen and an allogeneic HCT had been almost always fatal. Strategies utilizing noncytotoxic immunosuppressive conditioning regimens and HCT may salvage patients after rejection of the first graft.\textsuperscript{204} Aggressive medical management during the prolonged neutropenic period is required to prevent death from infection after transplantation of the second hematopoietic cell graft. Although the rates of rejection have been higher in the studies of nonmyeloablative HCT for hematologic malignancy (2% to 7%), graft rejection has not been associated with a significant morbidity or mortality because the duration of the pancytopenic period before autologous recovery has in general been short.\textsuperscript{172,205} When rejection occurs after reduced intensity conditioning, salvage HCT using a conditioning regimen of fludarabine and TBI (300 or 400 cGy) resulted in an engraftment rate of 87%.\textsuperscript{206} A different donor was used for salvage HCT in 63% of the cases. Nonrelapse mortality at 2 years was 24%. The estimated survivals at 2 and 4 years were 49% and 42%, respectively. Graft rejection can be successfully overcome by salvage HCT from the same or different donor with a reduced intensity conditioning regimen consisting of fludarabine and low-dose TBI.

**REGIMEN-RELATED TOXICITIES**

The severity of regimen-related toxicities is related to the intensity of the myeloablative therapy, the type of cytotoxic therapy, the medical condition of the patient before transplantation, and the presence of posttransplant factors, including the use of methotrexate, calcineurin inhibitors, and amphotericin B. Reduced intensity conditioning regimens may decrease the severity of certain regimen-related toxicities.\textsuperscript{188,207–210}

Oral mucositis occurs in more than 90% of patients after myeloablative conditioning, and most require pain relief with a continuous intravenous narcotic drug. Improvement occurs temporally, with narrow recovery approximately 2 to 3 weeks after transplantation. The severity of the oral mucositis depends on the intensity of myeloablative therapy and the use of methotrexate. Severe mucositis may result in upper airway obstruction or aspiration pneumonitis. A blinded randomized placebo control study of the recombinant human keratinocyte growth factor (palifermin) in patients receiving TBI plus high-dose chemotherapy followed by autologous stem cell rescue demonstrated a 35% reduction in severe mucositis and a 6-day reduction in the median time to recovery.
resorption of severe mucositis. Furthermore, palifermin was associated with decreased opioid and total parenteral nutrition use. The role of palifermin in other types of HCT recipients has not been established. Gastroenteritis induced by chemotherapy and irradiation results in nausea, vomiting, and diarrhea, all of which may persist for up to 2 to 3 weeks after transplantation. Central lines are placed prior to transplant for administration of fluids, drugs, and hyperalimentation during the period of therapy-induced mucositis and gastroenteritis.

Renal dysfunction is common after HCT. Some factors associated with the development of severe renal failure requiring dialysis were hyperbilirubinemia, significant fluid reduction after transplant, amphotericin B administration, and pretransplant serum creatinine levels greater than 0.7 mg/dl. Renal dysfunction (as indicated by a doubling of the baseline serum creatinine) was most strongly associated with the use of amphotericin B administration and SOS of the liver. In this study, calcineurin inhibitors, GVHD, sepsis, TBI, and CY were not found to be significant contributors to severe renal failure. Calcineurin inhibitors cause transient renal dysfunction that is generally reversible with aggressive hydration and eventual discontinuation of the drugs. Renal complications were decreased after HCT with a nonmyeloablative conditioning regimen.

The liver and lung are the two organs that limit further escalation of the intensity of myeloablative therapy. Idiopathic interstitial pneumonitis occurs in 8% to 18% of patients after myeloablative conditioning and marrow transplantation. Clinical manifestations include dyspnea, nonproductive cough, hypoxemia, and diffuse pulmonary infiltrates. This toxicity is most likely due to TBI, but preparative regimens containing high doses of chemotherapeutic agents such as CY, BU, and carmustine also contribute directly to toxicity in the lungs. Pulmonary veno-occlusive disease (VOD) is important to consider in patients with diffuse pulmonary infiltrates, since there may be a favorable response to corticosteroids. Treatment for idiopathic interstitial pneumonitis consists of supplemental oxygen and ventilatory support. Survival is poor (3%) for patients requiring intubation and mechanical ventilation for greater than 24 hours. Pulmonary complications are reduced after nonmyeloablative conditioning regimens.

SOS (formerly known as veno-occlusive disease, or VOD) of the liver is an event characterized by damage to vascular endothelial cells and to hepatocytes in zone 3 of the liver acinus and is the most frequent cause of hyperbilirubinemia within 20 days following HCT. Historically, the overall incidence of SOS was 53%, and 15% of patients had severe disease. The incidence of SOS has decreased in more recent years because of the reduced intensities of conditioning regimens and better selection of patients for myeloablative regimens. Risk factors for the development of severe SOS are pretransplant hepatitis with elevated serum levels of hepatocellular enzymes, the intensity of the conditioning regimen (TBI greater than 13 Gy), or the use of busulfan. Targeting strategies may have reduced the incidence of severe SOS in patients conditioned with busulfan. Patients receiving ursodeoxycholic acid after HCT had significantly lower rates of severe hyperbilirubinemia and elevated alanine aminotransferase levels, although the rates of SOS were similar compared to controls. Furthermore, the rates of severe GVHD were less and mortality was significantly improved at 1-year posttransplant in the ursodeoxycholic acid group. For the treatment of SOS, responses to the administration of tissue plasminogen activator have been reported. However, the risk of bleeding must be weighed against the potential benefit of this agent. Responses were also observed in patients treated with defibrotide which is a single-stranded polydeoxyribonucleotide with fibrinolytic and anti-thrombotic activity. No randomized clinical trials of defibrotide for treatment of SOS have been conducted. However, in a randomized clinical trial of prophylactic therapy, administration of defibrotide to pediatric patients undergoing HCT demonstrated decreased rates of SOS at day 30 when compared to controls.

Increasingly recognized as an important clinical problem are the delayed complications other than chronic GVHD after HCT (Chapter 106). Long-term risks associated with myeloablative conditioning include secondary malignancies, sterility, increased bone loss, delayed development in pediatric patients, neurocognitive changes, hypothyroidism, and cataracts. After autologous HCT there is an increased risk of myelodysplastic syndrome. Use of immunosuppression for chronic GVHD treatment was associated with an increased incidence of squamous cell cancer of the skin and oral cavity in allogeneic transplant recipients. Risk factors for defective spermatogenesis are older age at transplantation and presence of chronic GVHD. Rapid bone loss after transplantation can be partially corrected with the administration of bisphosphonate drugs. Children may experience delays in growth and development particularly after exposure to TBI-based conditioning regimens which may be improved with growth hormone therapy. Neurocognitive changes observed early after HCT largely recover at 1 year, however, full recovery after HCT, especially psychological recovery, may be a 3- to 5-year process. Recovery might be accelerated by more effective interventions to increase work-related capabilities, improve social support, and manage depression.

**Graft-Versus-Host Disease**

GVHD is mediated by genetically disparate lymphocytes after transfer to an immunologically compromised recipient incapable of rejecting the donor graft (Chapter 105). In the initial stages of GVHD, a donor T cell infiltrate is present and cell death occurs by apoptosis. In lymph nodes there is an absence of germinal centers which lasts for many months after HCT. Abnormal CD4:CD8 ratios can be found both in the lymph node as well as in the peripheral blood. The induction and release of signaling cytokines might be important in the development and severity of GVHD. The IL-10 pathway may play an important role in controlling the severity of acute GVHD. Plasma biomarkers for acute GVHD include IL-2 receptor-alpha, TNF-receptor-1, IL-8, and hepatocyte growth factor. Regenerating islet-derived 3 alpha (REG3 alpha), secreted by Paneth cells, is a plasma biomarker for severe acute GVHD of the gastrointestinal tract. In a multivariate analysis, stages 2 to 4 GVHD of the gastrointestinal tract, severe histologic damage, and high levels of REG3 alpha at GVHD diagnosis independently predicted nonrelapse mortality at 1 year.

GVHD has been traditionally classified into two different phases. Acute and chronic GVHD were defined as occurring before or after 3 months, respectively. However with increasing adoption...
of nonmyeloablative conditioning regimens in which engraftment of donor T cells occurs more gradually, the distinctions between these two phases of GVHD has blurred.\textsuperscript{189,254} Findings consistent with either diagnosis often occur concomitantly.

**Acute**

**Clinical Evaluation**

Acute GVHD involves three organ systems primarily—the skin, the gastrointestinal tract, and the liver. A maculopapular rash which can involve the palms and soles is often present and can be pruritic or painful. Bullae and epidermal separation may occur in severe cases and resembles toxic epidermal necrolysis. GVHD can also involve any part of the gastrointestinal tract. Symptoms and signs include nausea, vomiting, crampy abdominal pain, diarrhea, intestinal bleeding, and ileus. Lesions in the mouth may also occur. Patients may also present with persistent anorexia, nausea, and vomiting, and require endoscopy to evaluate the gastrointestinal tract for GVHD.\textsuperscript{255,256,257} Permanent scarring may occur with loss of mucosal regeneration and ulceration may persist for months. Lesions in the liver may be pruritic or painful. Bullae and epidermal separation may occur in very severe GVHD of the gastrointestinal tract.\textsuperscript{258} Cholestatic jaundice is common in liver GVHD but hepatic failure due solely to GVHD of the liver is unusual unless the GVHD is long-standing. A biopsy of these organs may be required to confirm the diagnosis of GVHD and to distinguish it from other posttransplant complications.

The major determinant for the development of acute GVHD is the degree of HLA match. The increased incidence of acute GVHD in HLA-matched unrelated donor transplants is likely attributable to increased disparity in mHC antigens or unrecognized disparities of the phenotypically matched major histocompatibility loci. Other factors influencing the rates of acute GVHD include sex mismatch with the donor, parity of female donors, TBI dose, and acute GVHD prophylaxis regimen.\textsuperscript{259,260,261} In peripheral blood HSC transplantation, the CD34-positive cell dose has been reported as an independent risk factor for acute GVHD.\textsuperscript{262}

To facilitate the study and prognostication of acute GVHD, a clinical staging and grading system (Glucksberg) was developed and then updated in 1995 (Chapter 105).\textsuperscript{263} Grade I or very limited acute GVHD has a favorable prognosis and does not require treatment. Therapy is usually required for grade II GVHD since it is moderately severe and usually affects multiple organs. Since patients with anorexia, nausea, and vomiting with positive biopsies for GVHD from the upper gastrointestinal tract usually require therapy, this was included as grade II. Grades III-IV GVHD are severe, affect multiple organs, and are associated with a decreased patient survival.\textsuperscript{259} An acute GVHD severity index was also developed, grouping patients with patterns of organ involvement associated with similar risks of treatment-related mortality and treatment failure.\textsuperscript{265} In a prospective evaluation of the two classifications, the performance was similar in explaining the variability in survival by acute GVHD grade.\textsuperscript{266} An acute GVHD activity index has also been developed.\textsuperscript{267} The activity index considers serum bilirubin, oral intake, treatment with prednisone, as well as performance, and may predict nonrelapse mortality better than either of the more commonly used clinical grading schema.

**Prevention**

The two major approaches to the prevention of acute GVHD after allogeneic marrow transplantation are pharmacologic immunosuppression and T-cell depletion. Agents for pharmacologic immunosuppression are generally more effective when used in combination. Methotrexate in combination with either cyclosporine or tacrolimus (both calcineurin inhibitors) are the pharmacologic agents used most commonly by transplant centers. In randomized controlled studies, the combination of tacrolimus and methotrexate was associated with a lower incidence of acute GVHD compared to cyclosporine and methotrexate, but there was no difference in the incidence of chronic GVHD or survival between the two groups (Fig. 102.7).\textsuperscript{268,269} These agents may have significant adverse effects and therefore patients must be monitored closely. Methotrexate delays, but does not prevent, hematopoietic engraftment. Methotrexate also potentiates the mucositis associated with myeloablative conditioning regimens. Nephrotoxicity and neurotoxicity are complications associated with the administration of cyclosporine and tacrolimus.

Other immunosuppressive agents including corticosteroids and MMF have been studied in combination with cyclosporine. The addition of prophylactic corticosteroids to the combination of cyclosporine and methotrexate or to cyclosporine alone had only limited if any benefit, and in one study a paradoxical increase in chronic GVHD was observed in the corticosteroid arm.\textsuperscript{270–272} MMF, a reversible inhibitor of inosine monophosphate dehydrogenase, has been successfully used in combination with cyclosporine for prevention of both GVHD and graft rejection after nonmyeloablative conditioning. The use of MMF instead of methotrexate in standard prophylaxis regimens after myeloablative conditioning regimens has been demonstrated to result in similar rates of acute GVHD, less mucositis, and faster neutrophil engraftment.\textsuperscript{273,274}

Alternative methods of GVHD prophylaxis include ex vivo T cell depletion of the hematopoietic cell graft, in vivo T cell depletion and posttransplant cyclophosphamide. Strategies for ex vivo T cell depletion include the use of monoclonal antibodies which...
are specific for T cells, selective agglutination to soybean lectin, and counter flow centrifugation elutriation. Although reduction in acute and chronic GVHD has been observed with transplantation of T cell-depleted marrow, there was an associated increase in graft rejection, infections, posttransplant lymphoproliferative disorders, and relapse. A randomized controlled trial did not demonstrate any survival benefits for ex vivo T cell depletion compared to standard pharmacologic immunosuppression for acute GVHD prevention after HCT from HLA-matched unrelated donors. In vivo T cell depletion with pretransplant ATG and alemtuzumab was also effective for reducing the risks of severe acute and chronic GVHD after reduced intensity conditioning. However, relapse rates were higher and overall survival was significantly decreased compared to the T cell replete group in the study. Relapses were not increased with in vivo T cell depletion if patients were conditioned with myeloablative regimens.

Posttransplant cyclophosphamide reduced the risks of both acute and chronic GVHD but until more experience has been obtained, it is not known if this approach will improve overall survival.

**Treatment**

The most common agents used to treat acute GVHD have been corticosteroids and ATG. In many marrow transplant centers the administration of corticosteroids (methylprednisolone or prednisone-equivalent dose of 2 mg/kg/day) had been the standard initial treatment for acute GVHD. In a retrospective analysis, it was observed that initial treatment with a lower dose of corticosteroids (methylprednisolone dose of 1 mg/kg/day) may be as effective as the higher dose. However, this needs to be confirmed in a prospective study. Higher doses of methylprednisolone (10 mg/kg/day) did not improve outcomes. Because complete remission of GVHD is seen in only 40% to 50% of the cases after initial or primary treatment, other immunosuppressive agents have been investigated in combination with prednisone. When a CD5-specific immunotoxin was added to prednisone for primary treatment, GVHD manifestations were more effectively controlled only during the first 5 weeks after start of treatment when compared to prednisone alone. Similarly, no long-term benefit was observed when ATG was added to prednisone. In a study of daclizumab in combination with prednisone as primary therapy, survival was significantly decreased compared to prednisone alone. This suggested that caution must be used with immunosuppressants that intensify immunosuppression for primary treatment of acute GVHD. In a randomized clinical trial of primary treatment for gastrointestinal GVHD, a short course of prednisone in combination with either placebo or beclomethasone dipropionate (BDP), an oral topical agent with limited systemic absorption, was evaluated. The use of BDP was associated with better control of upper gastrointestinal GVHD and improved survival at 200 days and 1 year posttransplantation. For steroid-refractory GVHD, immunosuppressive agents which have been studied include ATG, daclizumab, rapamycin, MMF, ABX-CBL (CD147-specific mAb), and pentostatin among others. Outcomes at 6 to 12 months after salvage therapy for steroid-resistant GVHD have been poor with a high transplant-related mortality. There are no standard effective therapies for steroid-refractory GVHD which can be recommended.

**Chronic**

**Clinical Evaluation**

Chronic GVHD is a syndrome that may develop as early as 50 to 60 days or as late as 400 days after transplantation. The incidence of chronic GVHD is dependent on the degree of disparity in the MHC antigens. It was observed in 33% of HLA-identical sibling transplants, 49% of HLA-nonidentical related transplants, and 64% of HLA-matched unrelated transplants. A prior history of acute GVHD is a significant risk factor for the development of chronic GVHD. Other risk factors for chronic GVHD are older age, female donor if male recipient, DLI, and use of peripheral blood stem cells. Corticosteroid use for GVHD prophylaxis may also increase the risk of the development of chronic GVHD.

Clinical features of chronic GVHD are skin lesions which may initially resemble lichen planus and may progress to generalized scleroderma, keratoconjunctivitis, oral mucositis, esophageal and vaginal strictures, intestinal abnormalities, chronic liver disease, pulmonary insufficiency secondary to bronchiolitis obliterans (BO), and a wasting syndrome. If generalized scleroderma occurs, it may lead to joint contractures and debility. Elevations in alkaline phosphatase and serum bilirubin are often the first indication of hepatic involvement with chronic GVHD. Bile duct damage has a similar histopathology as that seen in primary biliary cirrhosis. Liver biopsies are often helpful in establishing a diagnosis. BO affects the small airways of the lung and may occur at less than 150 days (40%) or more than 150 days (60%) following HCT. If serial pulmonary function tests show an evolving obstructive pattern, further testing is required including a high-resolution computed tomography scan to evaluate for the presence of BO. Keratoconjunctivitis sicca is a common but irreversible complication that is managed with frequent eye drops and tear duct ligation.

Screening studies and clinical manifestations of chronic GVHD are used in a clinical classification of chronic GVHD. Based on a retrospective clinical and pathologic review from 1980, a staging system was developed in which chronic GVHD was classified into limited and extensive. Extensive disease was associated with more frequent infections. The utilization of this original classification system was difficult because many patients were not easily classifiable by strict organ criteria and thus other significant prognostic factors have since been identified. Poor prognostic factors were extensive skin involvement, thrombocytopenia, and progressive-type onset. In 2005, the NIH consensus criteria were developed to standardize the criteria for the diagnosis of chronic GVHD. A clinical scoring system (0 to 3) described the extent and severity of chronic GVHD for each organ or site at any given time, taking functional impairment into account. A global assessment of chronic GVHD severity (i.e., mild, moderate, or severe) was then calculated based on the number of organs or sites involved and the degree of involvement in the affected organs. Diagnosis of chronic GVHD required at least 1 clinical sign or manifestation of chronic GVHD confirmed by biopsy or other relevant test and the exclusion of other possible diagnoses for those clinical symptoms considered to be chronic GVHD. Subtypes of late GVHD (>100 days) were recognized, including late onset acute GVHD, classical chronic GVHD, and an overlap syndrome (clinical symptoms of both acute and chronic GVHD).

**Prevention**

Several strategies to prevent the development of chronic GVHD have been pursued but none have made a significant impact on long-term overall survival. Prolonged administration (24-month course vs. 6-month course) of cyclosporine demonstrated no significant differences between the groups in the incidence of chronic GVHD, transplant-related survival, or overall survival. T cell depletion was associated with lower rates of chronic GVHD in HLA-matched sibling transplants, but not in the unrelated donor setting. No improvement in overall survival was associated with T cell depletion. Inclusion of alemtuzumab or ATG in the conditioning regimen was associated with a reduction in chronic GVHD.
Treatment

Extensive chronic GVHD that is left untreated has a poor prognosis. In general, based on the NIH consensus criteria, topical or local therapy is recommended for mild chronic GVHD, and systemic immunosuppression for moderate or severe chronic GVHD. In an original report of chronic GVHD, only 2 of 13 patients survived and there was the development of significant morbidity. Prednisone given for 9 to 12 months reversed many of the signs and symptoms of disease. The mortality in the group treated with prednisone was 21%. Patient survival was worse with the combination of azathioprine and prednisone compared to prednisone alone. The combination of cyclosporine and prednisone was first used in patients with high-risk chronic GVHD who had a platelet count less than 100,000. The results of this study appeared promising compared to historical controls. There was a trend toward improved survival and decreased morbidity. In contrast, a randomized study of combination therapy versus prednisone alone for standard-risk chronic GVHD suggested that more intense immunosuppression did not improve survival. A report of a randomized clinical trial in 2009 comparing MMF plus prednisone to prednisone only as primary treatment for chronic GVHD did not show any benefit. Standard practice for primary treatment of patients with moderate-severe chronic GVHD remains systemic immunosuppression with prednisone. There is no evidence that additional immunosuppression is beneficial. In practice, patients with extensive chronic GVHD are treated for a period of 9 to 12 months. At the end of this period, a reevaluation is done and, if there is no evidence of active chronic GVHD, the immunosuppressive agents are tapered. Other therapies which have been reported to have some limited efficacy for treatment of chronic GVHD are extracorporeal photopheresis for skin and oral GVHD and ursodeoxycholic acid treatment for GVHD of the liver. There is insufficient data to make strong recommendations regarding treatment of steroid-refractory chronic GVHD.

By 3 to 5 years after initiation of therapy, many patients have inactive chronic GVHD and immunosuppressive therapy has been discontinued (Fig. 102.8). Approximately 75% of patients treated for extensive chronic GVHD will survive with Karnofsky scores of greater than 80%. The major cause of excess mortality in patients with clinically extensive chronic GVHD is infections.

GRAFT-VERSUS-TUMOR EFFECT

Allogeneic immune surveillance or GVT reaction for malignancy contributes significantly to the therapeutic effectiveness of HCT. The term GVT refers to the GVL effect, the graft-versus-myeloma effect, as well as the effects on solid tumors by the graft. The first clinical report of a GVT effect after allogeneic marrow transplantation was published in 1979. It was observed that patients who developed GVHD had a lower risk of relapse after transplantation. In 1990, Kolb and colleagues successfully treated 3 patients with CML who relapsed after marrow transplantation with DLI from the original HLA-identical sibling marrow donor. In a subsequent report evaluating DLI in 135 patients with relapsed disease, complete remissions were achieved in 72% of CML patients and 29% of patients with AML. No responses were observed in patients with acute lymphoblastic leukemia. Chemotherapy to induce remission before DLI was a more effective strategy for long-term disease control for some patients with acute leukemia. GVHD and myelosuppression developed in 41% and 34% of patients, respectively, after DLI. Fourteen patients (10%) died from complications related to GVHD or myelosuppression. Another large study of 140 patients with relapsed malignancy after marrow transplantation confirmed the effectiveness and risks of DLI after relapse. Notably in both studies, GVHD after DLI correlated with disease response. The durability of the responses was confirmed with longer follow-up of patients who had achieved complete remission after DLI.

Figure 102.8: Prevalence of chronic graft versus host disease (GVHD) after allogeneic marrow transplantation from human leukocyte antigen (HLA)-identical sibling for aplastic anemia. The prevalence curve accounts for the time of onset of chronic GVHD and the time of its resolution in response to therapy. B: Probability of survival among the 29 patients with chronic GVHD (83%) and probability of discontinuing immunosuppression given for chronic GVHD (73%). Modified from Storb R, Blume KG, O’Donnell MR, et al. Cyclophosphamide and antithymocyte globulin to condition patients with aplastic anemia for allogeneic marrow transplantations: the experience in four centers. Biol Blood Marrow Transplant 2001;7:39–44. Used with permission.)

The number of CD3-positive cells infused (or DLI dose) is an important parameter in determining clinical response and risk for development of GVHD. Patients with CML had a lower risk of GVHD, and the efficacy of treatment was maintained with a strategy of escalating the dose of donor lymphocytes based on response of disease and development of GVHD. Comparable results with DLI from unrelated donors have also been demonstrated. The response, severity of GVHD, and the degree of myelosuppression were similar to that observed after DLI from HLA-matched sibling donors.

After nonmyeloablative conditioning and allogeneic HCT, the GVT effect is the main contributor to the disease response. Disease responses have been observed in both myeloid and lymphoid hematologic malignancies. Since the establishment of a GVT effect may not be achieved for 2 to 3 months after HCT, malignant disease needs to be controlled before HCT. Several retrospective studies have now shown comparable overall survival after either myeloablative or nonmyeloablative conditioning and HCT. However, all these studies showed relapse was higher and transplant-related mortality was lower after a nonmyeloablative conditioning regimen. In a randomized clinical trial of reduced intensity conditioning with TBI (800 cGy) and fludarabine compared to TBI (1,200 cGy) and cyclophosphamide in patients with AML, in first remission, there were no differences in transplant outcomes including relapse and nonrelapse mortality except for increased severity of oral mucositis in the TBI (1,200 cGy) group.

Although patients may have an excellent response to allogeneic HCT, severe GVHD may occur. Separation of GVHD from GVT...
effects may be possible because of disparate hematopoiesis-associated mHc molecules or tumor-restricted antigens on malignant cells. These tissue or tumor-restricted antigens could serve as targets for T cell immunotherapy to enhance GVTh activity without inducing GVHD. Peptides identified as minor histocompatibility molecules have been isolated and sequenced.332,333,334 Cytotoxic T lymphocytes (CTL) clones specific for recipient mHc antigens have been generated ex vivo.331 These were infused into 7 patients who had relapsed after HCT. Pulmonary toxicity was seen in three patients and in one it was severe. The CTL circulated for 21 days, and five patients had complete but transient remissions. The contribution of natural killer (NK) cells to the antitumor effect after allogeneic HCT has also been investigated.332 For AML, a disease with sensitivity to NK-mediated activity, NK cells from unrelated donors encoding the activating killer-cell immunoglobulin-like receptor KIR3DS1 were infused with improved relapse-free survival an HLA-C-dependent manner. These studies may help to explain the mechanisms associated with a GVT effect after allogeneic HCT, and in the future this information might be helpful for improving outcomes.

**IMMUNE RECONSTITUTION**

After myeloablative therapy and autologous or allogeneic transplant, both humoral and cellular immunity are impaired for months to years. After autologous HCT, NK cells in the peripheral blood recover by 1 month and B cells and CD8+ T cells recover by 3 to 6 months after HCT. However, CD4+ T cells are not restored to normal levels until 1 to 2 years after allogeneic transplantation, recovery of the immune system from the donor graft occurs in phases over a period of 1 to 2 years in patients who do not develop GVHD. There are significant delays in recovery of the immune system if GVHD develops.

The first phase of recovery is the increase in neutrophil counts which occurs 2 to 3 weeks after transplantation. Although the function of neutrophils is largely intact, impaired chemotaxis persists for a period up to 4 months.333 Monocyte numbers in the peripheral blood return to normal within 3 to 4 weeks after transplantation. Monocyte counts in the peripheral blood were inversely correlated with infection rates between days 100 and 365.333 Macrophages in the liver and lung have been shown to be of donor type by day 80. NK cells and other cell types capable of antibody-dependent cytotoxicity have recovered to normal levels by 30 days after transplantation. The recovery of the donor-derived immune system is important in the understanding of the GVT effect and complications after HCT, including graft rejection, GVHD, and infections.

**B Cells**

There are defects in serum immunoglobulin production initially after the transplant. Serum antibody responses to different antigens, including OKT74, keyhole limpet hemocyanin, pneumococcal antigen, and meningococcal antigen are lower than normal.337,338 The defect in antibody production occurs in both T cell–dependent and T cell–independent systems. B lymphocytes respond to mitogenic stimulation including staphylococcal aureus and cross-linked anti-IgM antibodies after 2 months. By 3 months after transplantation, B lymphocytes with surface immunoglobulin have recovered to normal levels. Persistent deficiencies in B lymphopoesis have been described in association with GVHD (acute or chronic) or its treatment.339 The number of B cell precursors in the marrow was not related to CD34 cell dose, type of transplant, donor age, or recipient age.

Serum IgG and IgM have typically achieved normal levels 1 year after transplantation in the absence of chronic GVHD.337 Serum IgA may remain low for a period of 2 years. If serum IgG levels are below 400 g/L, patients are generally treated with intravenous immunoglobulin.

**T Cells**

Reconstitution with donor T cells is poor initially in all recipients and remains defective in patients with chronic GVHD. Because of the faster recovery of CD8 positive T cells, a reversed CD4:CD8 ratio with low levels of CD4-positive cells has been demonstrated and persists in patients with chronic GVHD. In vivo cellular immunity, determined by skin testing to the recall antigens Candida, mumps, trichophyton, and the neo-antigen dinitrochlorobenzene, is diminished, but may be prolonged if chronic GVHD develops.341 In in vitro assays, donor T cells from patients have a decreased proliferation to mitogenic stimulus with phytohemagglutinin (PHA) and CD3-specific antibody. After 3 months the addition of exogenous IL-2 can normalize the proliferative response to PHA stimulation.342,343 By 6 months the response to PHA stimulation has normalized without the requirement for exogenous IL-2. T cell immunity to herpes simplex virus (HSV) can be detected by day 60.344 CTL function against varicella-zoster virus (VZV) and CMV is acquired later (≥3 months). Acquisition of T cell immunity to herpes virus may be delayed by prophylaxis of viral infections with anti-viral agents or GVHD treatment.345,346

Immunity early after myeloablative conditioning and allogeneic transplantation is restored by the adoptive transfer of mature T cells and may establish cellular immunity to infectious agents such as the herpes viruses.96,346-348 Vaccination protocols may also improve the pathogen-specific cellular immunity. An inactivated varicella vaccine given before, and during the first 90 days after, transplantation significantly reduced the risk for the development of varicella-zoster infections.349 CD4 T cell proliferation in response to the VZV was greater in patients who received the vaccine. Immune function after HCT may also be improved with the use of a peripheral blood stem cell graft. There were higher lymphocyte subset counts after HCT, and the rate of definite infections was significantly decreased compared to marrow.350 T cell–depleted HCT or therapy for active GVHD has been observed to delay the recovery of cellular immunity, thus resulting in an increased risk of infections. The thymus contributes to late immune reconstitution. T cell recovery is improved in younger patients compared to older patients, an affect attributable to the improved thymic function in the former group. Increased thymic output (i.e., high TREC levels) was associated with an increased number of naïve T cells and broader T cell repertoires, while low TREC levels correlated with the presence of chronic GVHD and severe opportunistic infections.351,352 Thymic rebound correlated with the capacity to respond to vaccinations.353 Measures to enhance thymic output after transplantation, especially in older patients, may enhance immune reconstitution and decrease the risk of infections.

**Web Sites**

The web site for the Center for International Blood and Marrow Transplantation is: www.cibmtr.org. This site provides more information on trends and survival data as well as published results of research studies.

**SELECTED REFERENCES**

The full reference list for this chapter can be found in the online version.


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