



Review

Prevention and treatment of acute graft-versus-host disease: the old and the new. A report from The Eastern Cooperative Oncology Group (ECOG)

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Summary:

There have been many advances in the prevention and treatment of GVHD, including cyclosporine, FK506, and combination therapies. This syndrome, however, continues to account for significant morbidity and mortality after allogeneic transplantation. With the expanded use of matched unrelated as well as mismatched related donors, the increase in incidence and severity of GVHD poses a new clinical challenge. Many of the newer agents discussed in this paper may have a role in the future as therapy for acute GVHD. The evaluation of these new agents and the approach to be taken is hampered by the realization that most patients have received and are relatively refractory to standard therapies. Clinical trials must be performed earlier in the course of the syndrome to establish the role of these compounds. Newer strategies are likely to include the use of sequential therapy directed at blocking endogenous cytokines followed by blocking alloreactive donor cells, and immunologic advances such as the induction of tolerance. What impact, if any, such therapy may have on amelioration of a graft-versus-leukemia effect remains unknown.

Keywords: graft-versus-host disease; cyclosporine; prednisone; transplantation; cytokines; monoclonal antibodies

Graft-versus-host disease (GVHD) continues to be a major complication after allogeneic bone marrow transplantation, even when the donor is a sibling who is genotypically identical at the major histocompatibility (HLA) locus and the recipient is given potent immunosuppression.¹⁻⁵ The GVHD reaction is manifest by erythroderma, generalized wasting, diarrhea, jaundice, and ultimately, death; the histopathological changes have been well-described.⁶⁻⁸

In clinical medicine, the GVHD syndrome has traditionally been subdivided into two syndromes: acute GVHD and chronic GVHD.⁹⁻¹² Acute GVHD develops in 30-60% of recipients of histocompatible sibling-matched allografts, and mortality due directly or indirectly to GVHD may reach 50%.¹³⁻¹⁵ Younger subjects, or those with younger donors, develop GVHD less frequently than older recipients of allogeneic bone marrow.¹⁶⁻²⁰ Even with the use of potent immune suppression, more than half the patients who receive unmanipulated marrow grafts from histocompatible donors develop GVHD; the risk is higher with histocompatible donors who are matched but unrelated, and histoincompatible (unmatched) yet related donors and the use of such alternative donors appears to be increasing.²¹⁻³⁰ Chronic GVHD develops in 35-50% of patients after transplant either as an extension of acute GVHD (progressive onset), after resolution of acute GVHD (quiescent onset), or without preceding acute GVHD (*de novo* onset).³¹⁻³⁶

GVHD in the human setting was hardly recognized when clinical transplantation was first undertaken. Subsequently, techniques of HLA typing and other advances have changed the approach to transplant. Over the past 25 years, the diagnosis, prophylaxis, and treatment of GVHD have evolved slowly, including the recognition that the GVHD syndrome is not entirely attributable to the immunologic response of major histocompatibility differences between donor and recipient. This review focuses on new clinical and experimental developments in the treatment of acute GVHD, examples of which are shown in Table 1. Although it is necessary to provide some discussion regarding therapy for chronic GVHD and prophylaxis of GVHD, these subjects will not be examined extensively.

Pathophysiology of GVHD

Acute GVHD initially was perceived as infiltration by immunoreactive donor T cells into target host tissues with resultant destruction. It has been over 25 years since Billingham proposed three essential requirements for development of GVHD: (1) the graft (donor cells) must contain immunologically competent cells; (2) the recipient (host) must express tissue antigens not present in the donor; and

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Table 1 Classes of agents for the treatment of GVHD

<i>Class of agent</i>	<i>Examples</i>
Non-specific immunosuppressive agent	Corticosteroids
Non-specific T cell immunosuppressives	Cyclosporine, FK506, rapamycin
Anti-cellular polyclonal antibodies	Antithymocyte globulin
Anti-cellular monoclonal antibodies	Anti-CD3; anti-CD12
Anti-cytokine monoclonal antibodies	Anti-IL-1 receptor antagonist; anti-TNF- α ; anti-IL-2 receptor antagonist
Anti-recognition monoclonal antibodies (anti-adhesion molecules)	Anti-LFA-1 α ; anti-MALA-2
Immunoconjugates (immunotoxins)	Anti-CD5-ricin; anti-IL-2-diphtheria toxin
Photopheresis	PUVA
Other agents	Thalidomide, desferrioxamine, penicillamine
Total lymphoid irradiation	—

(3) the recipient must be incapable of mounting an immune response to destroy the transplanted cells.^{8,37} Gowans³⁸ and McGregor³⁹ demonstrated that the cells responsible for the GVHD syndrome were small lymphocytes derived from allogeneic donor bone marrow. It has been shown that acute GVHD is initiated by donor-derived, alloreactive cytotoxic T lymphocytes (CTLs, CD8⁺ lymphocytes) and lymphokine-secreting T helper lymphocytes (HTLs, CD4⁺ lymphocytes).⁴⁰ The extent of histoincompatibility between donor and recipient, the presence of alloreactive, host-specific donor T cells, and the number of T lymphocytes present in the inoculum correlate with the incidence and severity of GVHD.^{41–43} Donor lymphocytes traditionally were thought to recognize differences in major host histocompatibility antigens leading to a graft-versus-host reaction. More recently, disparities in minor histocompatibility antigens between donor and recipient, inherited independently of HLA genes, were demonstrated to elicit a GVHD reaction.^{44–46} This reaction is then amplified by elaboration of cytokines (see below). Billingham's requirement for an immunoincompetent host has been revised recently, since GVHD has been described experimentally and clinically after infusion of either syngeneic (identical twin) bone marrow or autologous bone marrow.^{47–53} The recipient must either express tissue antigens not present in the donor, or the host must exhibit an inappropriate recognition of self-antigens, ie an autoimmune process.⁵⁴ In addition, immunohistochemical analysis of the effector cells in the GVHD reaction suggest these cells are natural killer cells rather than mature T cells.^{55,56}

The above data, taken together, lead to the concept of GVHD as a 'cytokine storm', defined as an outpouring of endogenous cytokines resulting in many tissue effects.^{57–60} Inflammatory cytokines such as tumor necrosis factor- α (TNF- α), and interleukin 1 (IL-1) are released during the preparative regimen and cytopenic phase of transplant; these agents incite both autoreactive and alloreactive inflammatory cells in a positive feedback loop, since inflammatory cytokines increase expression of class I and II MHC-peptide complexes, costimulatory ligands and adhesion molecules.^{61,62} The cytokines cause proliferation of effector cells, leading to more host tissue damage, further releasing cytokines. Many of the cytokines implicated in

the 'cytokine storm' reaction of GVHD are shown in Table 2.

GVHD can develop in the course of (1) bone marrow or peripheral blood progenitor (hematopoietic stem cell) transplantation, (2) transfusion of unirradiated blood products (transfusion-associated GVHD), or (3) solid-organ transplantation involving organs containing lymphoid tissue. This review will deal with the first two situations.

As understanding of the pathogenesis of GVHD has grown, therapeutic strategies have been modified.⁶³ It has been proposed that immunosuppression (either via T cell depletion of the graft and/or administration of immunosuppressive therapy to the host after the transplant, ie post-transplant immunosuppression) with its marked propensity for increasing opportunistic infections, be replaced by specific anti-cytokine therapy.^{64–67} Clinical trials have begun to address this question, although the efficacy of anti-cytokine therapy is still uncertain (see below).

Acute graft-versus-host disease

The clinical spectrum of GVHD was described clinically more than two decades ago.^{31,68–71} Acute GVHD usually becomes manifest within 20–40 days of marrow infusion, although potent immunosuppressive agents such as cyclosporine may delay onset by several months.⁷² Acute GVHD is graded clinically using a standardized system which takes

Table 2 Cytokines implicated in the pathogenesis of GVHD^{12,37,57,59,263,271,319,361–368}

IL-1
IL-2
IL-3
IL-4
IL-6
IL-11
TNF- α
Interferon-beta
Interferon-gamma
Lipopolysaccharide (endotoxin)
M-CSF

into account varying clinico-pathological involvement by different organs. While there is general acceptance of this system, there are minor differences among centers in the criteria used to assess the degree of organ involvement.^{31,73–77} The traditional method for grading acute GVHD was developed by Glucksberg and colleagues⁷⁷ in 1974, based on 61 patients. Recently, Rowlings and co-workers⁷⁸ compared the association of acute GVHD and transplant outcome in 2881 adult patients receiving HLA-identical sibling transplants for leukemia using a newly developed Severity Index which takes into account variability in prognosis within Glucksberg grades. They demonstrated the utility of this system in an independent patient population, and are comparing the Glucksberg grading system with the Severity Index in a prospective trial conducted by the French Society for Bone Marrow Transplantation.

Chronic graft-versus-host disease

Chronic GVHD presents a clinical pattern which usually differs from acute GVHD, ie mesenchymal rather than epithelial tissues are involved more commonly. Most classifications are based on the extent of disease and performance score.^{32,79} Chronic GVHD is a multi-organ disorder in which the severity of individual organ involvement does not correlate well with the overall survival; the patient's functional performance is a better indicator of survival.^{31,36} Chronic GVHD is categorized as either *limited* (localized skin involvement and/or hepatic dysfunction) or *extensive*; the latter is associated with a worse prognosis. Such a staging system, however, is not of much value since few patients have *limited* disease.

Understanding of the pathophysiology of chronic GVHD has lagged behind that for acute GVHD. While some investigators believe that chronic GVHD is merely a late expression of the alloreactivity causing acute GVHD, others postulate that chronic GVHD represents dysfunctional immune reconstitution through the generation of autoreactive clones.^{9,36,80} Data from experimental systems of autologous GVHD support this hypothesis since specific class II donor anti-donor autoreactive T cells can be demonstrated in both mice and humans who have chronic GVHD. This review will not address the therapy of chronic GVHD.

Transfusion-associated graft-versus-host disease

Transfusion-associated GVHD (TA-GVHD) is predominantly associated with infusion of unirradiated blood products.⁸¹ Historically, this syndrome was recognized over 20 years ago and the initial reports described its occurrence in children who had immunodeficiency disorders.^{82,83} Subsequently, this condition has been described in considerable detail, and approximately 150 patients have been reported in the literature.⁸⁴ Patients may be either immunocompromised, ie immunodeficiency disorder or hematologic malignancy, or immunocompetent, ie newborns. The syndrome usually occurs 4–30 days after transfusion of blood pro-

ducts and is characterized by a scaly, maculopapular, erythematous rash, high fever, diarrhea, and liver function abnormalities, as well as pancytopenia. As is the case with 'classic' acute GVHD, any of these features may present as the predominant clinical sign.

TA-GVHD often occurs after the transfusion of blood products shown by histocompatibility testing to be the same immunophenotype as the bone marrow and peripheral blood cell donor. Specifically, the clinical features are consistent with the host's inability to eliminate viable donor T lymphocytes present in the transfused blood product. For the syndrome to occur, the recipients ordinarily are HLA heterozygous for an HLA haplotype that is shared with an HLA homozygous donor. For a long time it was thought that the observed frequency of TA-GVHD was much lower than the estimated probability for this donor/recipient combination. Elegant mathematical models developed to estimate TA-GVHD risk, however, are in better agreement with the clinical experience.⁸⁵ Furthermore, it is likely that functioning recipient CD8 and NK cells play a central role in the down-regulation of TA-GVHD development in recipients.⁸⁶ Recent advances in therapeutic modalities, such as the potent T cell suppressor, fludarabine, may be responsible for the recent reports of TA-GVHD in chronic lymphocytic leukemia patients.^{87,88}

TA-GVHD has been reported after transfusion of any *cellular* blood product; blood components such as fresh frozen plasma and cryoprecipitate have not been associated with the syndrome.⁸⁴ Freezing removes almost all lymphocytes from the blood product, thus significantly minimizing the risk of initiating TA-GVHD. It must be emphasized that there is an extremely high fatality rate for established TA-GVHD, reaching 90% in some reports.⁸⁴ Because of the very high early mortality, chronic GVHD secondary to blood transfusion has only rarely been reported.⁸⁹

TA-GVHD usually develops in recipients given at least 10^7 lymphocytes per kg recipient weight,⁹⁰ although rare reports have implicated TA-GVHD with as few as 8×10^4 lymphocytes per kg.⁹¹ Although the condition is almost uniformly fatal in spite of therapy with high doses of corticosteroids and cyclosporine, there have been occasional reports of spontaneous resolution.⁹² Furthermore, experimental approaches using monoclonal antibodies such as anti-CD3 (OKT3) and cyclosporine have met with some success.⁹³

Fortunately, prevention of TA-GVHD is readily and most effectively accomplished using gamma-irradiation of cellular blood products.⁹⁴ A dose of at least 2500 cGy generally is adequate to inactivate donor lymphocytes and prevent TA-GVHD; this radiation dose does not significantly impair granulocyte and platelet function, although red cells do sustain detectable damage.⁹⁵ The 2500 cGy dose of gamma irradiation is remarkably effective,⁹⁶ although there have been rare reports of TA-GVHD in patients given irradiated blood products.^{97,98} It is estimated that 15% of institutions performing allogeneic or autologous bone marrow transplantation do not regularly irradiate cellular products for transplant recipients, probably due to unavailability of adequate irradiation facilities.⁹⁷ In general, bone marrow transplantation should only be performed at centers capable of irradiating all cellular blood components. Leuko-

cyte filtration devices can remove more than 99% of lymphocytes and are readily available; however, this method may not be as effective as gamma irradiation, since the residual lymphocyte 'load' can be associated with TA-GVHD after transfusion.⁹⁹

Prophylaxis of graft-versus-host disease

Concepts

Most preclinical modelling and clinical trials in GVHD have dealt with prophylaxis strategies. Effective prevention includes the use of histocompatible donors and recipients, the use of immunosuppressive drugs after the bone marrow infusion, the *in vitro* manipulation of the donor graft, and possibly housing the patient in a pathogen-poor, protected environment.¹⁰⁰ The agents in current use are much more efficacious in preventing the cascade of immunologic events we recognize as GVHD than in inactivating them once they are set in motion.

No GVHD prophylaxis

There have been only three small trials of transplants performed without GVHD prophylaxis and the results were conflicting.^{101–103} All three studies showed no difference in survival in treated *vs* untreated patients. One study showed no difference in incidence of GVHD, although both treated and untreated patients exhibited very high rates of GVHD;¹⁰¹ the other two studies found more GVHD in the untreated patients.^{102,103} Sullivan *et al*¹⁰³ reported 'hyperacute GVHD', ie severe, explosive GVHD, in patients not receiving prophylaxis. Although there is interest in avoiding immunosuppression in low-risk patients using pre-screening *in vitro* testing, at present there is no evidence on which to recommend the absence of GVHD prophylaxis in this group.¹⁰⁴

Two basic approaches to the prevention of GVHD have been utilized: post-transplant immunosuppressive drugs to modify immune responses in the host, and lymphocyte depletion of donor marrow. These two approaches will be considered separately.

Pharmacologic agents as GVHD prophylaxis

Corticosteroids and other lympholytic chemotherapeutic drugs were among the first agents examined in animal models and subsequently in clinical trials. The choice of agents and timing of administration was modified by the particular animal model used. Methotrexate was found to be the most potent agent in preventing and ameliorating GVHD in the canine model used by the group at the Fred Hutchinson Cancer Research Center, and hence they selected this agent for clinical use.¹⁰⁵ Cyclophosphamide, which had the most potent effects in the rat model of GVHD used at the Johns Hopkins Oncology Center was chosen for clinical use at that center.¹⁰⁶ Until the introduction of cyclosporine, methotrexate or cyclophosphamide given in low-dose pulses after the bone marrow infusion were the mainstays of GVHD prophylaxis. Both were associated with a 25–50%

incidence of GVHD, although cyclophosphamide was never compared directly to methotrexate. It is interesting to note, however, that none of these studies compared any of these agents to a 'no prophylaxis' arm.

The next agent used to prevent GVHD was anti-thymocyte globulin (ATG), which in some trials was effective in treating this condition. Two studies compared methotrexate prophylaxis to methotrexate plus ATG.^{107,108} These investigations showed no difference in incidence and severity of GVHD or overall survival. The University of Minnesota reported that ATG combined with methotrexate and prednisone reduced the incidence of GVHD from 48% (in patients receiving methotrexate alone) to 21%.²⁰ Overall survival and incidence of chronic GVHD, however, did not differ.

In the late 1970s the potent immunomodulator cyclosporine (cyclosporine A), known as ciclosporin in Europe, and cyclosporin in Australia and New Zealand, was introduced. Animal trials demonstrated this compound to be effective in preventing GVHD,¹⁰⁹ and several uncontrolled clinical trials indicated impressive results.^{110–116} Other trials, however, prospectively comparing cyclosporine to methotrexate prophylaxis therapy, showed no differences, although each agent was superior to a 'no prophylaxis' strategy.^{117–121}

The combination of cyclosporine and either methotrexate or methylprednisolone appeared to decrease the incidence of GVHD in several phase II and III studies.^{122–132} Storb and associates¹²² at the Fred Hutchinson Cancer Research Center reported that in severe aplastic anemia patients undergoing matched-sibling allogeneic bone marrow transplantation, prophylaxis with cyclosporine and methotrexate was associated with a significantly lower rate of grade II–IV acute GVHD than use of single-agent methotrexate, 18% *vs* 53%. Although actuarial survival at 2 years after transplant approached but did not reach statistical significance (82% *vs* 60%, $P = 0.062$), this group noted an increase in infections in the methotrexate group, no differences in the occurrence of chronic GVHD, and recommended combined therapy. In acute myeloid leukemia patients in first remission or chronic myeloid leukemia patients in chronic phase receiving cyclosporine plus methotrexate, the same group noted that the incidence of grade II–IV GVHD was 33% as compared to those given cyclosporine alone where the incidence was 54%.¹²⁶ A survival advantage was observed at 2 years in patients receiving combined cyclosporine and methotrexate. With additional follow-up, however, more relapses have occurred in the acute myeloid leukemia patients who received combined therapy, resulting in similar disease-free survival for both arms.^{127,128}

At the Johns Hopkins Oncology Center patients were randomly assigned in double-blind fashion to receive either cyclophosphamide and methylprednisolone, or cyclosporine and methylprednisolone.¹²⁹ The probability of developing acute GVHD was 68% in the cyclophosphamide arm and 32% in the cyclosporine arm; correspondingly, survival in the cyclosporine arm was double that in the cyclophosphamide group. Similar results have been reported in a phase II and a phase III trial.^{130,131} Shepherd *et al*¹³⁰ observed a 28.5% rate of grade II–IV acute GVHD after 28 matched-related allografts with combined cyclosporine and methylprednisolone; they indicated that renal

toxicity was avoided by the use of this phase II combination. The transplant team at the City of Hope reported a phase III study, in which patients were randomized to receive methotrexate plus prednisone or cyclosporine plus prednisone.¹³¹ Forty-seven percent of methotrexate-treated patients developed GVHD compared to 28% in the cyclosporine group. Correcting for crossover of patients who developed GVHD, a survival advantage was seen for cyclosporine-treated patients. In the three studies above, the incidence of chronic GVHD did not decrease despite improvements in the prevention of acute GVHD.^{122,126–131} Finally, in thalassemia patients undergoing allogeneic bone marrow transplantation, Galimberti and colleagues¹³² reported that the combination of cyclosporine, cyclophosphamide, and methotrexate was significantly more effective in preventing acute GVHD than cyclosporine used as single agent (41% vs 15%); event-free survival, however, did not differ in the two groups.

Table 3 illustrates the occurrence of acute and chronic GVHD after prophylaxis using single and multiple immunosuppressive agents. These pooled data reflect only randomized studies, and although the drugs are identical there were variations in dose and duration of therapy. Additionally, the type and stage of diseases for which the transplant was conducted varied. The pooled data may be useful to indicate the lower incidence of grade II–IV acute GVHD with the use of combined immunosuppressants, although the risk of relapse correspondingly is increased.

Thus, there is considerable evidence that cyclosporine, especially when given in combination with other agents, decreases the incidence and severity of acute GVHD after allogeneic bone marrow transplantation from histocompatible related donors. Although cyclosporine may have considerable side-effects, especially renal injury, and requires close monitoring of blood concentration, most bone marrow transplant teams currently use cyclosporine plus a brief course of methotrexate as their standard GVHD prophylaxis regimen.^{133–140} The use of cyclosporine as a continuous infusion has been reported by one group to facilitate dose monitoring and possibly improve efficacy, and reduction of the dose of methotrexate may improve compliance and reduce toxicity.^{141–142} On the other hand, combined cyclosporine and methotrexate appears to be less effective in unrelated donor transplants, and in situations in which the toxicities of the cytotoxic preparative regimen may preclude administration of full doses of cyclosporine and methotrexate.^{29,143–145}

One of the current controversies is whether adding corticosteroids provides added benefit to the combination of cyclosporine and methotrexate; the results of these trials differ.^{146–148} In one study, despite showing a benefit in reduction of the incidence and severity of GVHD, infectious complications in the corticosteroid-treated group were significantly higher, further emphasizing the delicate balance between immunomodulation and immunosuppression.¹⁴⁷ On-going clinical trials may clarify the relative efficacy of the three-drug vs two-drug prophylactic regimens.¹⁴⁹

Another agent to enjoy a brief period of interest was intravenous immunoglobulin (IVIG). IVIG has potential immunomodulatory and anti-infective effects. One trial suggested that patients at high-risk for GVHD, such as older patients sero-positive for cytomegalovirus, would benefit from IVIG therapy given prophylactically.¹⁵⁰ The high cost of this drug when given long-term and the negative results using survival as an endpoint in subsequent trials¹⁵¹ may have dampened enthusiasm for better defining its role and mechanism of action.

Phillips *et al*¹⁵² added the immunotoxin H65-RTA (anti-CD5-ricin A chain, see below) to a cyclosporine and methotrexate prophylaxis regimen in 31 patients receiving unrelated donor transplants, a group at extremely high risk for acute GVHD. All but two patients engrafted, and no late graft failures were observed. Grade III–IV acute GVHD developed in only seven patients. This prophylaxis strategy appears to reduce the severity of acute GVHD but requires confirmation in larger studies.

Limited data are available for another potent, specific immunomodulator, FK506 (tacrolimus).^{153–154} From a mechanistic viewpoint, there is no reason to anticipate that FK506 will have an advantage over cyclosporine.^{155,156} Differences exist, including the fact that hepatic metabolism of FK506 leads to higher concentrations in the liver, possibly explaining its advantage in allogeneic liver transplantation. One retrospective, single-center study, utilizing allogeneic hematopoietic stem cells collected from the peripheral blood rather than bone marrow, suggested that FK506 may be more effective than cyclosporine in preventing GVHD, but additional data are needed.¹⁵⁴

New agents that appear promising in animal models are succinyl acetone, 15-deoxyspergualin, leflunomide, and interleukin-12.^{157–161} Clinical usefulness of these agents may be limited by irreversible cerebellar toxicity with succinyl acetone and gastrointestinal toxicity with 15-deoxys-

Table 3 The occurrence of acute and chronic GVHD after prophylaxis using single and combination immunosuppressive agents

Agent(s)	No. patients	Grade II–IV acute GVHD %	Chronic GVHD (%)	Refs
MTX	143	45	27	31, 130, 122
CSA	50	54	24	128
MTX + CSA	128	27	38	122, 128, 146
MTX + CSA + Pred	122	26	45	146, 147
CSA + Pred or Methylpred	95	31	29	129, 131, 147

MTX = methotrexate; CSA = cyclosporine; Pred = prednisone; Methylpred = methylprednisolone.

These pooled data are taken from only randomized studies, and it must be appreciated that although the drugs are identical there may be variations in the precise doses and duration of therapy.

pergualin.^{157–159} Leflunomide was effective as prophylaxis and treatment of GVHD in transplantation of immunocompetent allogeneic cells into MHC-discordant F1 hybrid rats; clinical trials have not yet been reported.¹⁶⁰ Another agent, IL-12, appears to have promise in the prophylaxis of GVHD, although the only studies available to date are in mice.¹⁶¹ This cytokine, a potent immunostimulatory cytokine and inducer of cytotoxic T lymphocyte function, inhibited the development of GVHD with a single injection. Further studies of this paradoxical observation are warranted and will be noted with great interest. The reader is referred to other reviews addressing pharmacologic agents in the prevention of GVHD.^{162–164}

Lymphocyte depletion of the donor graft as GVHD prophylaxis

The second major approach to prevent GVHD is lymphocyte depletion to remove effector cells from the donor graft. The removal of lymphocytes as well as other accessory cells, however, can be associated with an increase in engraftment failure, or engraftment delay. On the other hand, as our understanding of GVHD has evolved, so has the appreciation that lymphocyte depletion may also remove many of the cells responsible for cytokines that mediate GVHD leading to disruption of the cytokine cascade and prevention/attenuation of the graft-versus-host reaction. This view is supported by the current interest in delayed infusion of donor lymphocytes, ie adoptive immunotherapy; this approach is associated with a potent graft-versus-leukemia effect and a lower likelihood and severity of GVHD.^{165–175}

A variety of techniques have been used to deplete lymphocytes from the donor graft, including monoclonal antibody, chemical, and physical separation methods.^{176–194} An overview is extremely difficult, since there is no standard approach. Relatively minor technical differences may make significant differences in clinical outcomes. Certain general principles, however, can be stated. All techniques are associated with a higher risk of engraftment failure, a complicated problem which may involve hematopoietic stem cell loss during marrow processing, lack of immunologic potency to overcome residual host defenses, or a combination. This is an on-going area of investigation, including the use of a novel, bone marrow-derived, engraftment 'facilitator cell' to compensate for increased risk of the lymphocyte depletion technique.^{195–197} This donor bone marrow cell expresses a unique immunophenotype (positive for CD8/CD3/CD45R/Thy 1/class II^{dim/intermediate} yet T cell receptor negative) and can facilitate engraftment in MHC-disparate allogeneic recipients without causing GVHD. In preclinical systems larger numbers of hematopoietic stem cells in the graft overcome this problem. In clinical situations, more intensive conditioning may be necessary. Finally, lymphocyte-depleted grafts are associated with higher relapse rates, particularly in chronic myeloid leukemia.¹⁹⁸ Weighing all of these factors makes comparisons of trials of lymphocyte depletion extremely difficult. This review will not deal with this technique in detail.

Cryopreservation of allogeneic bone marrow

Lasky and associates¹⁹⁹ previously reported successful allogeneic bone marrow transplantation in which the cellular product had been cryopreserved at the time of harvest. In a retrospective study one group compared the use of 10 matched, related allogeneic donor bone marrows which had been cryopreserved at the time of collection vs 33 matched related allogeneic bone marrow transplants which were infused fresh.²⁰⁰ They noted no differences in time to engraftment or blood product requirements, but the group given cryopreserved donor marrow had less acute GVHD. These results have been updated to include 49 patients who were given cryopreserved ($n = 31$) or fresh ($n = 16$) bone marrow donations; grade II–IV acute GVHD rates were 37% and 50%, respectively.²⁰¹ The reasons for this difference are unclear, but offer an interesting research opportunity in this field.

Conventional treatment of acute GVHD

Primary therapy of established GVHD

Despite prophylaxis, most patients develop acute and/or chronic GVHD. In the past, therapy for GVHD involved the same agents as used for prophylaxis.^{163,164,202–207} The use of these agents, including glucocorticoids, antithymocyte globulin, and cyclosporine, has been reviewed extensively.^{72,208–217} Early after transplant, before hematopoietic recovery, alloreactive T cells have not yet become fully expanded and differentiated. For this and other reasons, such cells can be eliminated or inactivated more easily by immunosuppressives. This finding may explain why these agents are more effective in preventing rather than treating established GVHD.³⁷

Despite a number of significant complications such as avascular necrosis of bone (relative risk 6.3, $P = 0.0002$), corticosteroids form the backbone of most regimens for treatment of acute GVHD.^{15,218,219} Weisdorf and co-workers¹⁵ reported the long-term outcome of 197 patients treated for grade II–IV acute GVHD among 469 patients who underwent allogeneic marrow transplant. Corticosteroid therapy alone was offered to 160 patients, while another 37 received other immunosuppressive agents. Complete response was attained in 72 patients (41%); another 61 required subsequent therapy with high-dose corticosteroids and/or antithymocyte globulin; 64 patients died from various causes. In a more recent study, this group reported that of 510 patients treated, the response to primary therapy was one of the most important predictors for long-term survival.²¹⁹

Some transplant centers begin with relatively low corticosteroid doses (ie 1 mg/kg/day prednisone equivalence) for patients presenting with limited cutaneous disease. High-dose glucocorticoid therapy usually is reserved for patients with systemic or severe skin disease. Although the dose is variable and ranges up to 3 g/day prednisone equivalence, many centers employ methylprednisolone in doses of 2.0–2.5 mg/kg/day. Although responses are obtained at very high doses, it is usually at the cost of severe catabolic damage, fluid retention, and

hyperglycemia. Furthermore, the use of high-dose corticosteroids will increase the risk of infection, but it is unclear to what extent. In one study in which corticosteroids were used as GVHD prophylaxis in the early post-transplant period, the risk of infection was increased by 50%;²²⁰ patients who have advanced GVHD are already at considerable risk for infection, and the benefit of controlling GVHD with corticosteroids may outweigh the drawbacks. After observing responses, corticosteroids generally are tapered every 4–7 days. Hings and co-workers²²¹ randomized 30 patients who had moderate or severe GVHD responding within 14 days to primary corticosteroid therapy to a slow vs fast taper. Short-course prednisone consisted of 2275 mg/m² administered over 86 days and the long taper regimen utilized 6300 mg/m² over 147 days. Patients given the brief course responded by 30 days (median), compared with 42 days in the other group ($P = 0.01$). Since the complications of corticosteroids, the incidence of chronic GVHD, and overall survival at 6 months were similar in both patient groups, these investigators favored a rapid taper.

Trials showing benefit of cyclosporine in treating acute GVHD have been limited to patients receiving non-cyclosporine containing regimens as prophylaxis. Patients who have not received cyclosporine as part of their GVHD prophylaxis may fare as well with cyclosporine treatment as with corticosteroid treatment.²⁰⁹

Combinations of various immunosuppressives have been used as GVHD treatment. ‘Triple therapy’ with prednisone, cyclosporine and anti-thymocyte globulin was found to be equally efficacious, but more toxic due to increased infections, than therapy with antithymocyte globulin plus cyclosporine alone.²¹⁰ Earlier, antithymocyte globulin and corticosteroids were found to be equivalent in methotrexate-treated patients.²¹⁵ The combination of prednisone and antithymocyte globulin was similarly ineffective in the treatment of acute GVHD occurring in the setting of unrelated bone marrow transplantation. Roy *et al*¹⁴⁴ reported a high failure rate in unrelated marrow transplant patients, since only nine of 42 patients responded to prednisone and antithymocyte globulin by 100 days after transplant. More recently, however, data for 267 HLA-A,B,D/DRB1-identical and minor mismatched unrelated transplants from the Fred Hutchinson Cancer Research Center showed no differences in non-relapse mortality or survival compared to matched, related transplants when adjusted for age and diagnosis.²²²

Secondary therapy of established GVHD

Initial treatment of GVHD remains unsatisfactory. Fewer than 50% of patients developing acute GVHD show durable improvement after initial treatment. Corticosteroid-resistant acute GVHD is extremely difficult to manage and is associated with high morbidity and mortality.^{15,144,218,223} One commonly used salvage drug is antithymocyte globulin, usually given in doses of 10–15 mg/kg every other day for 7–14 days. This agent provides effective anti-GVHD therapy in some patients but is quite toxic.^{210,214–216}

Martin *et al*²²³ retrospectively analyzed secondary therapy for acute GVHD in 427 patients without a durable response after primary treatment. Secondary treatment

included glucocorticoids ($n = 249$), cyclosporine ($n = 80$), antithymocyte globulin ($n = 114$), various monoclonal antibodies ($n = 19$), either singly ($n = 390$) or in combination ($n = 37$). Improvement or resolution was observed in 45% of patients with skin disease, 25% of patients with evaluable liver disease, and 35% of patients with evaluable gastrointestinal disease. Overall, complete or partial responses were noted in 40% of patients. The highest response rate was observed when GVHD recurred during the taper phase of glucocorticoid therapy. When this group was excluded, responses to secondary treatment were considerably fewer. These data led the authors to recommend evaluating new immunosuppressive agents in this setting with careful monitoring of the extent and severity of skin, intestinal, and liver organ dysfunction.

Newer approved agents

FK506

FK506 (tacrolimus) is a new drug that blocks T cell activation, similar to cyclosporine. In contrast to cyclosporine, FK506 does not affect natural killer cell function.²²⁴ FK506 was shown to have superior efficacy in preventing and treating rejection of transplanted livers in clinical trials.^{225–228} Similarly, in animal models FK506 is effective in preventing and treating GVHD.^{229–234} Published experiences using FK506 to treat GVHD were limited, until recently, to two reports.^{235,236} Koehler *et al*²³⁵ treated six children with acute GVHD resistant to corticosteroids. Improvement in acute GVHD was observed in skin and gastrointestinal GVHD in all six patients, and liver GVHD in three of four affected patients. Toxicity, however, was significant and similar to that described for solid-organ transplant patients treated with FK506.²²⁴ Five patients had renal toxicity, and two developed significant central nervous system injury. Kanamaru and associates²³⁶ studied 49 patients; 18 (13 evaluable) had acute and 31 (26 evaluable) had chronic GVHD. A ‘good’ to marked response was noted in seven of 13 patients in the acute GVHD group. In the chronic GVHD group, 10 had a ‘good’ and two a marked response. As expected, renal injury (53%) and nausea and vomiting (30%) were significant side-effects. These authors noted a correlation of toxicity with whole blood FK506 concentration, and recommended close monitoring for safety purposes. Further studies of this agent in the treatment of acute GVHD are warranted.

Investigational and experimental therapies for GVHD

Monoclonal antibody therapy

Attempts at treating GVHD by infusion of monoclonal antibodies *in vivo* have met with limited success.¹⁷⁶ Early trials utilized antibodies directed against the effector cells, such as anti-T cell antibodies. In recent years, knowledge gained from understanding of the role of cytokines in GVHD pathophysiology has fostered the use of antibodies against cytokines in experimental and clinical situations.

Anti-lymphocyte monoclonal antibody therapy

Reinherz *et al*²³⁷ administered a unique, non-mitogenic anti-CD12 monoclonal antibody to a patient with combined immunodeficiency who developed refractory acute GVHD after undergoing a haploidentical mismatched transplant; the GVHD resolved after antibody therapy. Other trials used a mitogenic anti-CD3 agent with mixed results.^{238–240} The cutaneous lesions in some patients responded to therapy, but only as long as the infusions were administered. In a dose-escalation study Martin and co-workers²⁴¹ treated 24 patients who developed GVHD with an IgG_{2a} anti-CD3 murine monoclonal antibody, 64.1. In two patients the antibody was used as initial therapy of GVHD while in 22 patients, it was given after failure of previous treatment. The antibody infusions were frequently associated with fever and chills. While skin GVHD in most patients responded to lower antibody doses, three-fold higher doses were required to suppress GVHD affecting liver and intestine. No patients attained a complete response, and all needed additional immunosuppressive therapy. Furthermore, four patients developed Epstein–Barr virus (EBV)-associated lymphoproliferative disorders within 7–18 days after initiating monoclonal antibody therapy.^{238,241} These mitogenic anti-CD3 antibodies, combined with the severe immunosuppressive agents and the immunodeficiency of GVHD, abrogated the T cell-mediated surveillance mechanism that normally modulates the proliferation of EBV-infected lymphocytes.

Several investigators reported the successful use of non-mitogenic anti-T cell antibodies without the development of lymphoproliferative disorders.^{242–244} Remlinger *et al*²⁴³ used four monoclonal anti-T cell antibodies that reacted with mature T cells (pan T cell antibodies) to treat corticosteroid-resistant acute GVHD. In this phase I study, six of 10 patients receiving intermediate to high doses of antibody appeared to have some improvement in GVHD.

Anasetti and colleagues²⁴⁴ reported the results of a phase I–II study with a murine IgG_{2b} non-mitogenic anti-CD3 monoclonal antibody, termed BC3. Fourteen patients with GVHD were given BC3 at a dose of 0.1–0.2 mg/kg/day for 7 or 8 days. Side-effects included mild chills, fever, hypertension, and chest discomfort. Eight patients responded, five completely and three partially. Although responses were sustained in most patients and four of the complete responders had effects lasting longer than 1 year, most patients died of infection.

Hebart and colleagues²⁴⁵ reported results of treatment with two murine monoclonal antibodies in 14 patients who developed severe corticosteroid-resistant GVHD. These antibodies included OKT3, an IgG_{2a} directed against one of the CD3 subunit complexes, and BMA031, an IgG_{2b} directed against an epitope in the constant region of the human α/β T cell receptor. Seven patients were given OKT3 as a single agent, five for acute GVHD and two for chronic GVHD. The first injections were associated with fever, chills, tachycardia, dyspnea, and hypotension. Three of five who received therapy for grade II–IV acute GVHD responded completely. One of the responders (grade IV GVHD) died of aspergillosis; the two others with grade II and III GVHD remain alive, free of GVHD 628+ and 720+

days after transplant. One of the two patients given OKT3 for extensive chronic GVHD had a partial response, but both died (one due to aspergillosis and the other due to chronic GVHD). BMA therapy was not associated with any toxicities. However, none of the seven patients given BMA treatment for grade II–III acute GVHD responded completely, although five had partial responses lasting 51 to 1461+ days. Only one patient developed anti-isotypic or anti-idiotypic antibodies. Fatal infections occurred in several patients. Neither agent appeared to have a sufficiently good therapeutic index to justify routine use.

Recently, Heslop and colleagues²⁴⁶ reported encouraging results with CBL-1, the murine IgM monoclonal antibody directed against activated T cells and natural killer cells, which has been effective in reversing graft rejection in renal allografts.^{247,248} Nine of ten patients with grade III–IV corticosteroid-resistant GVHD responded within 2 weeks of treatment; five subjects had complete resolution, and marked response was noted in four others. The effect was durable, since only one partial and one complete responder had recurrence of acute GVHD. Although several patients died of relapse or subsequent infection, four subjects remain alive 89+ to 1173+ days after therapy (three with chronic GVHD).

Anti-cytokine antibodies in treatment of GVHD

As early as 1971, it was postulated that endogenous cytokines played a role in GVHD.²⁴⁹ Donor T lymphocytes appeared to be essential for GVHD; the development of syngeneic and autologous GVHD, however, ultimately led to a realization that IL-1, TNF- α , IL-2, and other cytokines were extensively involved in the pathogenesis of GVHD reaction.^{250–253} As a result, some of the most promising results in the treatment of GVHD appear to be with the newer monoclonal antibodies directed against the cytokines (TNF- α and IL-1), or the cytokine receptors involved in the GVHD reaction (IL-1 soluble receptor, IL-2 soluble receptor).

IL-1 and antagonists

IL-1 is a central cytokine in the activation of T cells and in the pathogenesis of GVHD.²⁵⁴ IL-1 production occurs early in the inflammatory process and leads to increased expression of TNF- α and other cytokines.^{255,256} Abhyankar *et al*²⁵⁷ used competitive polymerase chain reaction technique to measure cytokine mRNA transcripts in GVHD target organs. They noted that in the setting of GVHD, IL-1 mRNA was elevated several hundred-fold in the skin and other target organs, while TNF- α transcripts increased only four- to six-fold, and IL-2 transcripts were elevated only transiently and slightly. While these data are interesting, they must be interpreted with caution in view of the competitive assay technique used to provide quantitation. In mice, McIntyre and colleagues²⁵⁸ infused the recombinant human IL-1 receptor antagonist and 35F5, a neutralizing monoclonal antibody to the type I mouse IL-1 receptor and blocked the local accumulation of neutrophils and the acute inflammatory responses to intraperitoneal injections of IL-1 and lipopolysaccharide.

Both Abhyankar *et al*²⁵⁷ and McCarthy *et al*²⁵⁹ demonstrated in mice transplanted with bone marrow exhibiting minor histocompatibility differences that IL-1 receptor antagonists were effective both as prophylaxis and treatment of GVHD. Hematopoietic reconstitution was not affected and mortality from GVHD was reduced. Recent data from a preclinical mouse model, however, were disappointing, since IL-1 receptor antagonists did not completely inhibit GVHD when donor bone marrow was fully MHC disparate.²⁶⁰ Protection was significant but only partial despite the use of extremely high doses. The authors concluded that intervention with a single cytokine antagonist might be insufficient to render a lasting protective effect and urged caution in the conduct of clinical trials using a similar design.

Since IL-1 receptor antagonists were shown to have some efficacy in experimental animal systems, various clinical trials were initiated. Antin and co-workers²⁶¹ conducted a phase I/II trial to evaluate the effectiveness of an IL-1 receptor antagonist in 16 patients who had severe, corticosteroid-resistant GVHD. They infused antibody as a 24-h continuous infusion over 7 days, and escalated the dose from 400 to 3200 mg/day. Improvement was noted in the skin (8/14), gastrointestinal tract (9/11), and liver (2/11). Lack of response of hepatic GVHD may reflect difficulties in measuring response in the liver, differential responses, or other hepatic toxicities during marrow transplantation such as veno-occlusive disease and drug toxicity. Acute GVHD was improved by at least one grade in 10 of 16 patients. In addition, the improvement in acute GVHD co-occurred with a reduction in TNF- α mRNA levels in blood mononuclear cells. The toxicity profile of the IL-1 receptor antagonist was acceptable, the only adverse effect being reversible serum transaminase elevation in two patients. These data corroborate the data generated in experimental animal systems that IL-1 is a mediator of GVHD. Although acute GVHD did not resolve in all patients, the results of this phase I/II study are important and may justify a larger trial using the maximal effective dose of drug.

Tumor necrosis factor and antagonists

TNF- α is a macrophage-derived cytokine with multistimulatory effects on T cells and an important mediator of the inflammatory process. TNF- α has also been implicated in the pathogenesis of GVHD.^{262–266} TNF- α infusion mimics most manifestations of GVHD in experimental animal systems.²⁶⁴ In one preclinical study polyclonal or monoclonal anti-TNF- α antibody prevented GVHD,²⁶⁴ but in another²⁶⁰ TNF- α antagonists, even at high doses, were not effective. In two studies Dickinson *et al*^{267,268} used an *in vitro* skin explant model to test the role of different cytokines in development of GVHD. They showed that supernatant from mixed lymphocyte cultures contained factors which induced features in the skin grafts identical to those seen in GVHD *in vivo*. Antibodies to TNF- α and interferon (IFN)- γ reduced these changes. They also demonstrated marked synergy between TNF- α and interferon- γ , as has been demonstrated in other situations.²⁶⁹ Loetscher²⁷⁰ demonstrated that a recombinant TNF- α receptor can

inhibit circulating TNF- α and IL-1 although it has not yet been demonstrated to inhibit tissue-derived agents *in vivo*. Holler *et al*^{271,272} retrospectively described elevated serum levels of TNF- α in patients who developed acute GVHD and other complications such as capillary-leak syndrome and hepatic veno-occlusive disease. In a prospective study of over 100 patients, increased serum TNF- α correlated with increased severity of GVHD and other problems such as hepatic veno-occlusive disease.⁶¹ Equally important, the timing of the elevation in serum TNF- α predicted subsequent development of severe complications and overall survival, ie those patients who developed increases before marrow infusion as a result of the preparative regimen had a 90% chance of developing GVHD and fewer than 30% of these patients survived. Although a causal relationship between TNF- α and GVHD has not been established unequivocally, such high-risk patients theoretically could benefit from anti-TNF- α antibody infusions. This approach is currently being investigated in prospective trials.

Several trials address the use of anti-TNF- α monoclonal antibodies in the treatment of GVHD. Hervé and associates^{273–277} studied the murine IgG₁ monoclonal antibody (B-C7) in 24 patients with resistant grade III–IV GVHD. Patients were given 0.1–0.4 mg/kg antibody in six doses over 8 days and no side-effects were observed. Although there were no complete responses, 17 patients had a partial response. Gastrointestinal and skin GVHD responded best, but some patients experienced improvement in hepatic GVHD. GVHD recurred (in all but three patients) a median of 3 days (range: 2–120 days) after discontinuing therapy. These three patients with a sustained response remain alive 7, 13 and 15 months after transplant. Four other patients remain alive, but most patients died from GVHD. Holler *et al*^{272,278} reported preliminary results of infusing either B-C7 ($n = 22$) or MAK 195 F, a murine F(ab)₂ fragment of an anti-TNF- α antibody ($n = 8$) in resistant GVHD. Therapy was well tolerated, rapid improvement was noted in affected organs in two-thirds to three-quarters of patients, but symptoms recurred quickly after cessation of therapy. These studies indicate that TNF- α is an effector of human acute GVHD. However, they also demonstrate that modulation of cytokines alone may be insufficient to interrupt ongoing immunologic activation in advanced GVHD. Results might be better if reagents such as anti-TNF- α are used earlier in the course of GVHD or prophylactically, or in combination with immune suppressive agents.²⁷²

IL-2 and antagonists

IL-2 also appears to play a role in the pathogenesis of GVHD. Theobald *et al*²⁷⁹ demonstrated that the number of donor T cells that secrete IL-2 in response to host antigens will accurately predict development of GVHD after a histocompatible sibling-matched transplant. Alessandrine *et al*²⁸⁰ and Siegert *et al*²⁸¹ found a correlation between incidence of GVHD and raised serum concentrations of soluble IL-2 receptor complex. The immunosuppressive activity of such antibodies may reflect both blocking of IL-2 binding and IL-2-dependent T cell proliferation as well as destruction of IL-2 receptor-positive cells.²⁸² Since soluble cytokine receptors or antagonists can be cloned, it may be poss-

ible competitively to inhibit the biological activity of the cytokine by preventing IL-2 from reaching its receptor, without developing neutralizing antibodies. This strategy may also be useful for receptors for IL-4 and TNF- α , which also have been cloned recently.

Two clinical studies evaluated the benefits of prophylaxis with an antibody to soluble IL-2 receptor. Belanger *et al*^{283–284} administered the anti-IL-2 receptor monoclonal antibody 33B31 to 64 patients undergoing matched, unrelated allogeneic marrow transplantation, along with methotrexate and cyclosporine. No toxic effects were noted but neither were there differences in incidence and time of onset of severe GVHD, occurrence of chronic GVHD, engraftment, relapse, or survival when compared to a historical control group of 89 patients. Tiley and co-workers²⁸⁵ treated 31 allograft patients with GVHD using a murine monoclonal IgG₁ anti-IL-2 receptor antibody (B-B10, also known as BT 563). Thirty patients received matched-sibling marrow, and one received marrow from a matched unrelated donor. Seventeen patients responded completely and six partially, but GVHD recurred within a median of 10 days after completion of anti-IL-2 receptor antibody therapy in 12 patients.

Cuthbert and colleagues²⁸⁶ treated 14 corticosteroid-resistant patients with a murine monoclonal anti-IL-2 receptor antibody (termed BT 563, also known as B-B10) which reacts with the p55 epitope. No toxic effects directly attributable to the antibody were noted. Eight patients responded, four attaining a complete and four a partial response. One complete responder developed chronic GVHD and died of infection 4 months after therapy. Three complete responders who had grade III or IV GVHD, however, remain alive without GVHD 4, 5 and 7 months after therapy. Cahn and co-workers²⁸⁷ completed a double-blinded, placebo-controlled, multicenter trial using the BT 563 monoclonal anti-IL-2 receptor antibody to treat 69 patients who developed grade II and III GVHD. No statistically significant differences were observed between the two groups during or upon completion of therapy in GVHD grade or probability of survival at 1 year.

Based on a successful preclinical animal model in which Ferrara *et al*²⁸⁸ reduced the severity of GVHD by injections of an anti-IL-2 receptor monoclonal antibody, several clinical GVHD prophylaxis trials have been done. Blaise *et al*^{289–291} reported their results using 33B31, a rat IgG_{2a} anti-IL-2 receptor monoclonal antibody in genotypically identical and one antigen-mismatched sibling bone marrow transplant patients. All patients received GVHD prophylaxis with cyclosporine, 'short course' methotrexate, and daily intravenous infusions of anti-CD25 monoclonal antibody. No major adverse effects were noted and engraftment did not appear to be delayed. No human anti-rat antibodies were noted. Grade II–III GVHD developed in four patients 32–40 days after marrow infusion. These disappointing results with prophylactic use of this antibody suggest it to be of limited value.

In 1988 Hervé *et al*²⁹² reported that a monoclonal antibody against the IL-2 receptor was effective as therapy for established GVHD in 10 patients. Subsequently, 58 patients who developed corticosteroid-resistant GVHD after a sibling-matched allograft were treated with an IgG₁ murine

monoclonal antibody (B-B10) directed against the IL-2 receptor.^{277,293} The patient population included 31 patients with grade II GVHD, 21 with grade III GVHD, and six with grade IV GVHD. In the course of this phase I–II study patients were given 75–100 mg antibody over 20 days without any toxic effects reported. Twenty-nine of 58 patients (50%) had complete resolution of GVHD; these responses included 19/31 with grade II GVHD, 8/21 grade III GVHD and 2/6 grade IV GVHD. An additional 12 patients achieved a partial response for a total of 41 responders in 58 treated patients; 17 of these patients (41%) had recurrence of GVHD. Twenty-six of 58 remain alive 240–900 days after antibody therapy, while 16 have died from GVHD-related causes. The investigators have indicated that a phase III trial evaluating this antibody is underway. In addition, these investigators reported the use of the B-B10 antibody in 25 partially matched and matched-unrelated transplant patients who developed GVHD.²⁷⁷ The patient population included 14 patients with grade II GVHD, nine with grade III GVHD and two with grade IV GVHD. Complete resolution of GVHD was observed in 15 patients ($n = 10$ grade II; $n = 4$ grade III; $n = 2$ grade IV GVHD). Another six patients exhibited a partial response to therapy, for a total of 21 of 25 responders (88%). GVHD, however, recurred within 2–30 days after cessation of antibody therapy in nine of the 21 responders. Thirteen patients remain alive, while 15 died from GVHD-related causes; the best results occurred in those patients given the B-B10 antibody earlier in the course of their GVHD.

Hervé *et al*^{275,276} reported in preliminary fashion the use of the B-B10 monoclonal antibody in 99 heterogeneous allogeneic transplant patients who had steroid-resistant GVHD. Similar data were noted as above, since GVHD recurred in nearly half the cases within 2–30 days after discontinuation of monoclonal antibody therapy. Also, GVHD affecting the liver responded poorly to this therapy. This reagent, however, has definite activity in the treatment of GVHD, especially if therapy is instituted soon after onset. In addition, monitoring of other parameters such as soluble CD8 and TNF- α predict a positive response to anti-IL-2 therapy.²⁹⁴ These studies suggest a role for this antibody in combination therapy.

Anasetti and co-workers^{295,296} reported the use of several preparations of anti-IL-2 receptor antibodies. They reported the use of 2A3, a murine IgG₁ monoclonal antibody specific for the IL-2 binding site (CD25) in 11 corticosteroid-refractory patients.²⁹⁵ Antibody was given as 0.1–1.0 mg/kg/day for 7 days. Toxic effects included fever, chills, respiratory distress, hypertension, hypotension, and four patients developed human anti-mouse antibodies. Anti-GVHD responses were seen in four patients: a complete response in one patient who had skin only disease, and partial responses in three patients in the skin and intestine. No responses were observed in the liver and only one of the responding patients survived more than 2 months from the start of antibody treatment. These modest results may reflect either the severity of the disease or the development of human anti-mouse neutralizing antibodies.

Advances in technology allowed the development of a more fully humanized, less immunogenic monoclonal antibody, termed anti-TAC.²⁹⁷ Humanized anti-TAC is a gen-

etically engineered human IgG₁ monoclonal antibody specific for the IL-2 receptor β chain (CD25) which blocks the IL-2-dependent activation of human T lymphocytes. Twenty patients who had failed corticosteroids as primary therapy for GVHD were treated with single doses of antibody in cohorts of 0.5, 1.0 or 1.5 mg/kg antibody; responding patients could receive additional antibody therapy.^{296,298} Therapy was well tolerated since only chills (one patient) and diaphoresis (one patient) were noted during or shortly after antibody infusion. No patients developed a measurable antibody response to therapy. Improvement in GVHD was noted in eight patients (four complete and four partial responses), including amelioration in three of 15 patients with GVHD affecting the liver. One complete responder did not develop chronic GVHD and is alive 645 days later off all immunosuppressives; the seven other responders later developed chronic GVHD, and one patient is alive 529 days later.

Immunotoxins

Immunoconjugates have been used in a variety of situations as therapy for GVHD and have been reviewed.²⁹⁹ After the report of successful treatment using one agent, H65-RTA, (XomaZyme; XOMA Corporation, Berkeley, CA, USA), a more extensive experience has been reported.³⁰⁰ This immunoconjugate was the first drug of its type introduced for clinical immunosuppression. It was developed to combine an anti-CD5 murine monoclonal antibody, which will react with an antigen present on 95% of peripheral T cells, with ricin A chain, a potent toxin. A phase I trial was conducted in 34 patients who had corticosteroid-refractory acute GVHD.³⁰¹ Nine patients attained a complete response, while seven achieved partial responses. No patients died of GVHD within the first 100 days after therapy, and the duration of response was for at least 100 days. Skin and intestinal GVHD responded most favorably, but some patients experienced improvement in liver GVHD. Circulating lymphocyte numbers decreased with drug administration but returned to baseline with cessation of the infusions. A variety of side-effects were reported, including fever, chills, arthralgias, depression, lethargy, fatigue, transient hypoalbuminemia, and weight gain. While renal dysfunction was observed, this toxic effect could not be linked directly to the immunoconjugate, since most patients were already receiving nephrotoxic agents. Interestingly, while six patients developed human anti-mouse antibodies, in no instance did these agents interfere with binding of the immunoconjugate to CD5 cells, and no allergic reactions developed. Subsequent trials comparing this agent to historical controls have been reported in preliminary fashion.^{302,303} Seventy-five corticosteroid-refractory patients were given H65-RTA and 18 (24%) patients attained a complete response, while 21 (28%) had a partial response of GVHD. While responses occurred in all affected organs, the skin responses were twice as frequent as those noted in the liver, and intestinal GVHD results were midway in frequency. Overall, 39 of 75 patients (52%) responded to this approach, whereas only 31% of a matched historical control population responded to antithymocyte globulin treatment. Similarly, the median survival was better in the

treated group (148 days) compared to only 80 days in the historical control group. Despite these results this agent has not been approved for use in bone marrow transplantation by the US Food and Drug Administration.

Combination anti-lymphocyte and anti-cytokine monoclonal antibody therapy

One group devised a novel strategy in which three monoclonal antibodies were used in sequence as therapy for corticosteroid-resistant acute GVHD.³⁰⁴ The murine IgG₁ anti-TNF- α monoclonal antibody (B-C7) was given for 4 days along with the murine IgG_{2b} anti-CD2 (B-E2) monoclonal antibody (derived against human thymocytes) for 10 days, followed by the murine IgG₁ monoclonal anti-IL-2r antibody B-B10 (renamed BT563). Fifteen patients who had steroid-resistant acute GVHD underwent therapy, including six subjects who had grade II GVHD, two grade III, and seven grade IV disease. Five patients had a complete response while four experienced a significant but incomplete response to therapy. GVHD, however, recurred in four of these responding patients despite continued treatment. This group demonstrated the tolerance of multiple monoclonal antibody therapy and the potential of combining antibodies that have differing mechanisms of action.

Thalidomide (N-phthalidoglutarimide)

Thalidomide was originally developed as a sedative but was observed to have anti-inflammatory and immunosuppressive properties; in 1961 this agent was withdrawn from clinical use because of its teratogenic effects.^{305,306} Recently, thalidomide has received attention as a treatment for GVHD.³⁰⁷⁻³¹¹ Thalidomide exerts its action via several mechanisms, including direct immunosuppressive properties and inhibition of TNF- α production as well as the induction of antigen-specific suppressor cells.^{307,308,312-314} Thalidomide therapy has been associated with sedation in virtually all patients treated; some patients develop a peripheral neuropathy which resolves with cessation of drug. Thalidomide has been most effective in the treatment of chronic rather than acute GVHD.³¹⁵⁻³¹⁸

Interferons

IFN-gamma appears to play a role in GVHD. Smith *et al*³¹⁹ used a mouse model to demonstrate that IFN-gamma is a mediator of the immunosuppressive activity of GVHD of the spleen. Niederwieser and colleagues³²⁰ noted that endogenous levels of IFN-gamma preceded the clinical onset of GVHD. Furthermore, in a murine model, antibodies to IFN-gamma provided protection to the gut.³²¹ Thus, based on these initial preclinical results, there may be justification for considering anti-IFN-gamma antibodies in expanded animal studies, and possibly in a prospective clinical trial.

Nitric oxide

Nitric oxide appears to be a short-lived biological mediator that plays an important role in host defense and macrophage

function.^{322,323} Various inhibitors of nitric oxide synthesis, such as L-N^Gmonomethyl arginine have been tested in pre-clinical systems to examine the role of this intermediary agent. Garside *et al*³²⁴ showed in a haploidentical allogeneic transplant animal model that an inhibitor of nitric oxide synthetase, L-N^Gmonomethyl arginine, reduces the gastrointestinal pathology of GVHD in a dose-dependent manner. Drobyski *et al*³²⁵ used another murine allogeneic minor histoincompatibility transplant model to evaluate the effect of the same nitric oxide synthase inhibitor. Amelioration of GVHD by inhibition of nitric oxide synthase early after transplant was suggested by their data, but this was not conclusive. On the other hand, use of L-N^Gmonomethyl arginine adversely affected alloengraftment, which would be a great concern were this approach to be used in a clinical situation. Additional animal trials are needed before considering the use of this agent in clinical trials.

Anti-adhesion molecules

Recent work has suggested that blockade of cell adhesion molecules may effectively block cell/cell interactions, thereby interfering with recognition, signaling, and other facets of the GVHD reaction at the cellular level. Harning and associates³²⁶ studied two antibodies to the cellular adhesion molecules LFA-1 α (CD11a) and MALA-2 (the murine homologue of ICAM-1). Mice given anti-LFA-1 α antibody (M17/4.2) or anti-MALA-2 (YN1/1.7) experienced significantly reduced GVHD and enhanced survival. LeDeist *et al*³²⁷ transplanted 10 leukocyte adhesion deficiency patients using T cell depleted HLA-non-identical transplants, yet graft rejection did not occur. These results suggested that host T cells and natural killer cells, which lack the LFA-1 antigen, poorly interact with donor marrow cells. Van Dijken *et al*³²⁸ administered five daily infusions of anti-LFA-1 antibody early after transplant to mice given incompatible T cell-depleted marrow and demonstrated a low incidence of engraftment failure. These experimental data indicate that use of anti-adhesion molecules may have a role in either facilitating engraftment in high-risk situations for engraftment failures, or in abrogating GVHD.

Photopheresis therapy of GVHD

Several groups have used photopheresis in the treatment of GVHD.^{329–337} *Ex vivo* treatment of patient blood after *in vivo* treatment with 8-methoxypsoralen inactivates immunocompetent lymphocytes to diminish the manifestation of GVHD. This approach has often been employed in patients with GVHD limited to skin.^{329–337} The sample sizes have usually been small, but many of patients responded to this intervention. In one of the larger series, Deeg *et al*³³⁵ reported their experiences in 18 patients who failed to respond to prednisone alone or in combination with an anti-CD5 immunotoxin ($n = 13$), or antithymocyte globulin ($n = 5$). Patients were given 1–32 treatments with 8-methoxypsoralen followed by ultraviolet A irradiation (PUVA). Ten patients responded and did not require additional therapy for acute GVHD. In a recent study, Aubin and co-workers³³⁷ reported 11 patients with acute and chronic GVHD predominantly of skin, which was

resistant to standard as well as innovative immunotherapy. Three of the four patients who had acute GVHD exhibited complete clearing of skin manifestations. Such promising results suggest a greater role for this agent in the treatment of acute GVHD.

New agents

Some of the newest and more novel agents which could or have shown promise in the treatment of GVHD are listed in Table 4.

Rapamycin

Rapamycin, originally discovered as an antifungal agent, is an immunosuppressive drug similar to cyclosporine and FK-506 although its action occurs later in T cell activation. It inhibits T cell activation by interfering with signals induced by the T cell growth factors IL-2 and IL-4.^{338,339} In a recent report Quesniaux and colleagues³⁴⁰ showed *in vitro* that this agent blocks the proliferative responses of a number of cell lines and bone marrow progenitors to many cytokines, including IL-1, IL-3, IL-6, IL-11, G-CSF, GM-CSF, and stem cell factor. While they noted that rapamycin suppressed hematopoietic recovery in mice given cytotoxic drugs, it had no effect on myelopoiesis in normal mice. In a murine experimental model, rapamycin prolonged the survival of recipients of allogeneic donor grafts when administered during the GVHD process.³⁴¹ This agent appears to be a general inhibitor of cytokine-driven proliferation and ultimately may be too toxic for use in patients who have recently undergone high-dose therapy. It may be useful, however, in treating GVHD in some subjects in whom the bone marrow appears to function adequately, or in whom regimen-related toxicity is not a problem.

Class II peptide binding molecules

Antigen-specific T cell activation occurs when the T cell receptor recognizes antigenic peptide fragments bound to self molecules encoded by the major histocompatibility complex (MHC).³⁴² CD8⁺ T cells recognize peptides complexed to class I MHC molecules, and CD4⁺ T cells recognize peptides in association with class II MHC molecules.³⁴³ It has been possible to design molecules which interfere with the MHC-peptide-T cell-receptor triad, resulting in prevention of distinct T cell-mediated autoimmune diseases.^{344–346} Based, in part, on the work cited above, Schlegel *et al*³⁴³ showed that the administration of peptides (myelin basic protein residues) with high binding affinity for class II MHC molecules prevented GVHD in mice undergoing transplantation across minor histocompatibility barriers. This approach may be potentially useful for the prevention and treatment of GVHD in allogeneic bone marrow transplant patients.

Table 4 Examples of newer immunosuppressive agents and mechanism of action^{158,229,338–341,369–374}

<i>Agent</i>	<i>Proposed mechanism of action</i>
FK-506	Inhibition cytokine synthesis
Rapamycin	Inhibition cytokine action
Humanized anti-CD25 monoclonal antibody	Cellular cytokine receptor blockade
Anti-IL-1 receptor antagonist	Cellular cytokine receptor blockade
Anti-IL-2 receptor antagonist	Cellular cytokine receptor blockade
Anti-TNF- α	Serologic cytokine blockade
Mycophenolic acid	DNA synthesis inhibition
Deoxyspergualin	Inhibition cell maturation
Brequinar	Dihydro-orotate dehydrogenase/DNA synthesis inhibition
Leflunomide	Inhibition cytokine action
L-N ^G monomethyl arginine	Inhibitor of nitric oxide synthase
Anti-cell adhesion molecules (anti-LFA-1)	Inhibition cell–cell interactions
Class II peptide-binding molecules	Inhibition MHC dual recognition

Other agents

2-Chlorodeoxyadenosine

Pless and colleagues³⁴⁷ reported the unsuccessful use of 2-chlorodeoxyadenosine in two patients who had corticosteroid-refractory GVHD. The infusion was well-tolerated but significant myelosuppression occurred in both patients. Both patients died of GVHD without response to 2-chlorodeoxyadenosine. Both deaths were from intestinal GVHD 11 and 49 days after starting therapy. Considering the potent and sustained immunosuppressive effects of this agent, it is doubtful that 2-chlorodeoxyadenosine will play a significant role in the therapy of established acute GVHD.

Desferrioxamine

The iron chelator desferrioxamine inhibits IL-2 receptor expression on phytohemagglutinin-stimulated lymphocytes *in vitro*. In a preliminary communication Michallet and associates³⁴⁸ reported the results of desferrioxamine used as therapy for GVHD in conjunction with methylprednisolone. Seventeen of 18 patients with skin GVHD responded; six of nine with intestinal and seven of 10 with liver GVHD responded. Reversible ototoxicity and ocular toxicity was noted, along with transient thrombocytopenia. This novel approach may merit further consideration in prospective, randomized fashion.

Penicillamine

Summerfield and co-workers³⁴⁹ reported a patient who received penicillamine for sclerodermatous chronic GVHD. The syndrome was previously resistant to both corticosteroids and azathioprine. After treatment with penicillamine, skin ulcers and joint contractures resolved completely. The report of a successful outcome in one patient who received penicillamine therapy may be sufficient justification for further study.

Anti-interleukin-2/diphtheria toxin conjugate

Kelley and associates³⁵⁰ used diphtheria toxin conjugated to a rat anti-mouse IgM IL-2 receptor antibody to suppress a variety of T cell-mediated reactions in mice. They noted that this immunotoxin blocked delayed-type hypersensitivity and selectively eliminated the targeted T cells from draining lymph nodes. Kuzel *et al*³⁵¹ reported a phase I study in which the diphtheria IL-2 fusion protein DAB₄₈₆IL-2 was used as an anti-tumor agent against mycosis fungoides and non-Hodgkin's lymphomas. Although untested to date, this strategy has the potential to be useful in the treatment of GVHD in humans.

Mycophenolic acid

This agent, which is a potent and specific inhibitor of the synthesis of guanosine nucleotides and thus a selective suppressor of proliferation of both T and B lymphocytes, has been used for the prevention of rejection in human renal and liver transplantation.^{352–355} This agent has not yet been used to any great extent in clinical allogeneic bone marrow transplant trials, but may receive added interest in view of its unique mechanism of action and extensive experience in solid-organ transplantation.

IL-10

This cytokine possesses both anti-inflammatory as well as immunosuppressive properties, in part by inhibiting production of IFN-gamma and TNF- α .^{356,357} In a parental-to-F₁ mouse model, IL-10 did not prevent lethal GVHD but reduced clinical manifestations due to cytokine mediators such as IFN-gamma and TNF- α .³⁵⁸ Clinical trials using IL-10 in allogeneic transplantation have not yet been initiated but will be watched with great interest.

15-Deoxyspergualin

This agent is a potent, immunosuppressive antibiotic derived from *Bacillus laterosporus* culture supernatant.³⁵⁹ Kasai *et al*³⁶⁰ described a case report of a patient with cyclosporine- and corticosteroid-resistant, endoscopically proven intestinal GVHD who responded to 15-deoxyspergualin therapy. Endoscopic examination revealed dramatic improvement in the mucosal lesions, and he remains without recurrence 20 months after therapy. This striking response in the intestine merits additional evaluation of this agent in the allogeneic transplant setting.

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