Biology of Blood and Marrow Transplantation 10:655-668 (2004)

1083-8791/04/1010-0001$30.00/0
doi:10.1016/j.bbmt.2004.07.007

Novel Approaches to the Therapy of Steroid-Resistant Acute Graft-versus-Host Disease

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Received May 13, 2004; accepted July 30, 2004

INTRODUCTION

Acute graft-versus-host disease (GVHD) is a principal impediment to the cure of patients with blood disorders after allogeneic stem cell transplantation. Thus, much attention has been paid to understanding the pathophysiology of GVHD with the hope that elucidation of the mechanisms of GVHD will improve our ability to both prevent and treat this complex problem. The working framework for allogeneic GVHD is derived from Billingham and Brent's initial postulate that the development of GVHD requires that the graft contain immunologically competent cells and that the host contain membrane antigens lacking in the graft, which can be recognized as foreign [1]. We now generally accept that donor T cells are the immunologically competent cells and that these T cells recognize a set of host polypeptides as foreign. These antigens include both major and minor histocompatibility antigens and are thought to be metabolic products of normal protein metabolism that are processed through the endoplasmic reticulum and displayed by HLA molecules. These polypeptides are polymorphic and lead to T-cell recognition, activation, and, ultimately, tissue injury through a variety of cellular effector mechanisms. In essence, the donor's resting immune system suddenly comes in contact with new antigens in the setting of tissue injury that accompanies conditioning regimens, infection, and the underlying disease. Direct cell-mediated attack, production of inflammatory mediators (such as tumor necrosis factor alpha [TNF]–α and interferon gamma [IFN]–γ), and recruitment of secondary effectors completes the accelerating cycle of cell injury and inflammation that we recognize as GVHD [2]. In this setting, the donor's immune system now finds itself in a milieu of upregulated chemokines, cytokines, and adhesion molecules, precisely as if there were a serious systemic infection [2,3]. Therefore it follows that if a therapeutic intervention can interrupt the GVHD, it may also interfere with the recipient's ability to respond to infection. Thus, control of established GVHD should be associated with an increased risk of opportunistic infections.

Corticosteroids have been the primary therapy for acute GVHD for more than 3 decades [4]. Standard treatment of GVHD with high-dose corticosteroids effects a durable response in approximately 40% of patients after histocompatible sibling transplantation [5] and in only 24% after unrelated donor transplantation [6]. Thus, 60% to 75% of patients who develop clinically significant GVHD will require therapy beyond corticosteroids. For patients with steroid-refractory disease, no standard effective treatments are available [7]. A variety of agents have been studied in this setting, but outcome is generally disappointing. In this report, we review treatment of steroid-resistant GVHD, including studies of newly available agents.

ANTITHYMOCYTE GLOBULIN

The problems with the management of steroid-refractory acute GVHD are perhaps best illustrated in the experience with antithymocyte globulin (ATG). ATG has been a staple of GVHD therapy for many years and remains the most commonly used secondary treatment, especially among pediatric transplant centers [8]. A number of studies have demonstrated that

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ATG has activity in steroid-refractory GVHD, with 19% to 56% responses overall and skin responses from 59% to 79% [5-16]. However, overall survival has not improved, and 1-year mortality approaches 90%. Deaths typically are not from GVHD per se but are primarily from opportunistic infections that follow the prolonged and global immunosuppression. In concordance with this, Arai et al. [14] demonstrated that survival was similarly poor for patients regardless of their GVHD severity at the time of starting ATG, although a report from another group did show an improvement in survival when ATG was given as part of early therapy [15]. The use of ATG is also complicated by the fact that at least 14 different formulations of horse- and rabbit-derived products are currently available worldwide. Furthermore, different brands, or even different lots of the same brand, of ATG may contain varying titers of antibodies against T-cell antigens, and this yields unpredictable bioactivity. The relative efficacy of horse- versus rabbit-derived ATG has never been compared in the stem cell transplantation setting. Finally, a standard dose and schedule for ATG in the treatment of GVHD has not been established, and an alarming observation was revealed by a recent international practice survey: only 6% to 28% of the 153 responding transplant centers were using ATG dosing guidelines published in the literature [8].

ANTI–INTERLEUKIN-2 RECEPTOR MONOCLONAL ANTIBODIES

Because activated T cells play a major role in both the initiation and maintenance of GVHD, it was logical to target cells bearing the interleukin (IL)–2 receptor (IL-2R). This rationale is based on the principle that T-cell activation and expansion under the influence of IL-2 is crucial in the pathogenesis of acute GVHD. The human IL-2R is a heteromeric complex composed of up to 3 polypeptide chains, designated as α-, β-, and γ-subunits. On the basis of the combination of these subunits, the receptor exists in low, medium, and high IL-2 affinity binding forms. Most resting lymphocytes and natural killer (NK) cells express on their surface an intermediate-affinity IL-2R, which contains the β- and γ-chains, and expression of the α-subunit (CD25) is often restricted to T cells after antigen stimulation. The limited distribution of CD25 on activated lymphocyte subsets suggests that monoclonal antibodies against this subunit may be used to deplete alloreactive T cells in patients with GVHD. In the early 1990s, murine anti–IL-2R monoclonal antibodies were tested for steroid-refractory acute GVHD in human clinical trials. Complete response (CR) rates of 66% and 73% were reported in human trials of the murine monoclonal antibodies B-B10 and BT563, respectively [17,18].

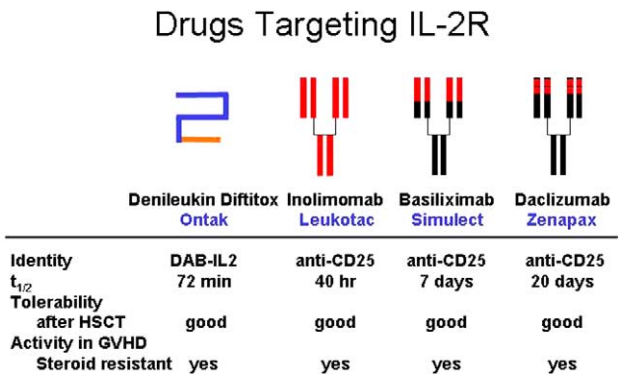

Figure 1. Available agents targeting human IL-2R: agents targeting activated T cells through the IL-2 receptor. For the 3 monoclonal antibodies, red indicates murine structural elements, and red is the humanized portion. For denileukin diftitox, gray represents the IL-2 molecule, and black represents the diphtheria toxin moiety. Comp/ADCC, complement activation/antibody-dependent cellular cytotoxicity; RE-blockade, reticuloendothelial blockade. Figure is adapted courtesy of Dr. Fred LeMaistre.

The notion that these reagents may delete only activated T cells while sparing resting T cells suggested that immunologic recovery might be enhanced compared with a pan–T-cell antibody such as ATG. Several monoclonal antibodies with varying human and mouse elements are available (Figure 1). Most studies in hematopoietic stem cell transplantation were performed with daclizumab (Zenapax; Roche Pharmaceuticals, Nutley, NJ), a 144-kd humanized immunoglobulin G1 monoclonal antibody that binds specifically to the α-subunit (p55 α, CD25, or Tac subunit) of the human IL-2R, where it inhibits IL-2 binding. Daclizumab has been used extensively in solid organ transplantation, where it seems to decrease the number and severity of rejection episodes without increasing adverse events, infectious complications, or late malignancies [19,20]. Daclizumab demonstrated a 40% response rate in a phase I trial of steroid-refractory acute GVHD [21]. A subsequent larger trial reported a CR rate of 47% when daclizumab was administered twice weekly, whereas overall survival was 53% at 4 months [22]. Encouraging activity has also been reported in steroid-refractory GVHD for other commercially available IL-2Ra antibodies, including inolimomab (BT563) and basiliximab [18,23,24]. Laboratory correlate studies revealed significant reductions in $CD3^+CD25^+$ lymphocytes after daclizumab treatment, although these reductions did not correlate with clinical response. On the basis of these encouraging results, a multicenter randomized, double-blinded, placebo-controlled trial testing the addition of daclizumab to corticosteroids as initial therapy for acute GVHD has recently been completed. Unfortunately, there was significantly inferior 100-day survival (77% versus 94%; $P = .02$) and overall survival (29% versus 60%; $P = .002$) when daclizumab was com-

bined with corticosteroids, despite an equivalent rate of GVHD control (53% versus 51%; $P = .85$) [25].

DENILEUKIN DIFTITOX

Denileukin diftitox (Ontak; Ligand Pharmaceuticals, San Diego, CA) is a recombinant fusion protein with selective cytotoxicity against activated T lymphocytes based on its preferential binding to high-affinity IL-2R. Denileukin diftitox consists of an N-terminal methionine, the first 386 amino acids of diphtheria toxin, fused to amino acid residues 1 to 133 of IL-2. After internalization by IL-2R–mediated endocytosis, the diphtheria toxin portion of the molecule is cleaved, and the catalytic A fragment is transferred to the cytosol, where it catalyzes adenosine diphosphate ribosylation of elongation factor 2 on ribosomes, halts cellular protein synthesis, and triggers programmed cell death [26].

Pharmcokinetics of Denileukin Diftitox

Aside from its selectivity for activated T cells, denileukin diftitox has pharmacokinetic properties that may render it favorable for use in patients with GVHD. Denileukin diftitox is metabolized by ubiquitous proteolytic degradation, and dose adjustments are not necessary for hepatic or renal dysfunction. After administration, denileukin diftitox follows dose-proportional kinetics and exhibits monophasic clearance from the serum, with a terminal half-life of 72 minutes. A phase I study in lymphoma patients has also established that the serum concentration of denileukin diftitox does not accumulate despite repeated daily administrations [27]. This pharmacokinetic profile may have important implications in terms of minimizing prolonged immune suppression.

Denileukin diftitox may also be advantageous compared with monoclonal antibodies directed against CD25 on the basis of its mechanism of action. Unlike monoclonal antibodies, which function by blocking IL-2 binding to the IL-2R and depend on secondary effector mechanisms such as complement activation or antibody-dependent cellular cytotoxicity for cell kill, denileukin diftitox exerts its cytotoxic effects directly via its toxin moiety. The kinetics of this cytotoxicity are extremely efficient. In vitro studies suggest that only 10 to 40 binding sites are necessary on the cell surface for entry, and that entry of only 1 molecule of denileukin diftitox is sufficient to induce cell death [28].

Finally, it has been suggested that that bioactivity of anti-CD25 monoclonal antibodies could be dampened by binding to soluble IL-2R (sIL-2R), a phenomenon known as *cold-target inhibition* [29]. However, concentrations of purified sIL-2R as high as 2500 pmol/L had no effect on the in vitro biologic activity of denileukin diftitox. Similarly, LeMaistre et al. [30] reported that the presence of sIL-2R did not prevent antitumor responses mediated by DAB_{486}IL-2, a precursor molecule to denileukin diftitox. These results suggest that sIL-2R competes poorly with denileukin diftitox for the native IL-2R [27,30].

Denileukin Diftitox in Acute GVHD

Ho et al. [31] conducted a phase I study investigating denileukin diftitox in 32 patients with steroid-refractory acute GVHD. Three dose schedules were evaluated: level 1, 9 μg/kg intravenously on days 1 and 15; level 2, 9 μg/kg on days 1, 3, 5, 15, 17, and 19; and level 3, 9 μg/kg on days 1 to 5 and 15 to 19. Dose escalation was determined by dose-limiting toxicity (DLT) at each dose level. After the maximum tolerated dose (MTD) was determined, 10 additional patients were enrolled to assess efficacy. The initial dose of denileukin diftitox was administered over 60 minutes, with subsequent doses over 30 minutes. Patients were premedicated with diphenhydramine, acetaminophen, and corticosteroid. Whenever possible, the daily steroid dose was used as premedication.

At dose level 3, all 4 evaluable patients developed DLT (1 renal failure and 3 hepatic transaminase elevation). Therefore, dose level 2 was considered the MTD. Hepatic dysfunction, defined as alanine aminotransferase or aspartate aminotransferase ≥5 times baseline or the upper limit of normal or total bilirubin ≥3 times baseline, was the most common DLT (30%), occurring in 4 (22%) of 18 patients at the MTD. Although increased hepatic transaminase was common in the week after denileukin diftitox administration, isolated hyperbilirubinemia was rare and was observed in only 1 patient in this trial. Other severe adverse events potentially attributable to therapy included infusional reaction, acute renal failure, cardiac tamponade, and sepsis, but these toxicities occurred in very few patients and were difficult to ascribe to therapy per se. Severe vascular leak syndrome was not observed, and no patient developed respiratory distress from pulmonary edema. Most patients had some degree of peripheral edema and hypoalbuminemia before and after therapy with denileukin diftitox. Eight of the 32 patients died during the study period. Causes of death were GVHD, sepsis/multiorgan failure, idiopathic pneumonia syndrome, intracranial hemorrhage, and liver failure.

GVHD responses to denileukin diftitox are shown in Table 1. Of the 24 patients evaluable for GVHD response, 8 (33%) resolved all evidence of GVHD (CR) on or before study day 29, and 9 (38%) improved by at least 1 grade (partial response; PR), for an overall response rate of 71%. Of the PRs, 4 subsequently entered a CR without additional immune-suppressive

Table 1. *GVHD Response to Denileukin Diftitox*

Variable	Complete Response	Partial Response	Overall Response
Dose			
Level 1	1/7 (14%)	4/7 (57%)	5/7 (71%)
Level 2	6/13 (46%)	3/13 (23%)	9/13 (69%)
Level 3*	1/4 (25%)	2/4 (50%)	3/4 (75%)
Overall	8/24 (33%)	9/24 (38%)	17/24 (71%)
Organs involved			
Skin	7/16 (44%)	4/16 (25%)	11/16 (69%)
Intestine	9/16 (56%)	3/16 (19%)	12/16 (75%)
Liver	1 (25%)	0	1 (25%)
GVHD grade at enrollment			
II	1/8 (13%)	4/8 (50%)	5/8 (63%)
III	5/13 (38%)	4/13 (31%)	9/13 (69%)
IV	2/3 (67%)	1/3 (33%)	3/3 (100%)

*Only 1 patient at this level completed the intended 10 doses, because of toxicity; 4 of 9 PRs converted to CR after day 29 without additional therapy.

therapy, resulting in an overall CR rate of 50% (12/24). GVHD response correlated with increasing dose frequency. The best responses were observed at dose level 2, at which 6 (46%) of 13 achieved a CR and 3 (23%) had a PR that subsequently converted to a CR after 1 month (resulting in an overall CR rate at level 2 of 69%). GVHD responses to denileukin diftitox were substantial in patients with skin and intestine involvement, with individual organ CRs of 44% and 56%, respectively, and overall response rates of 69% to 71%. Ten patients had previously been unsuccessfully treated with daclizumab, and it is interesting to note that 8 of these patients either completely or partially resolved the GVHD after denileukin diftitox (1 CR and 7 PRs). With extended follow-up, 9 of 30 patients treated are alive (median, 7.2 months). Among the 12 patients who ultimately had complete resolution of GVHD, 7 (58%) are alive. Conversely, only 1 of 12 evaluable patients who did not achieve a CR is alive ($P < .001$). There have been 5 late deaths among patients in CR: 3 from infection (2 bacterial and 1 fungal), 1 from chronic GVHD, and 1 from relapse. No Epstein-Barr virus–associated lymphoma or cytomegalovirus disease has been observed.

Flow cytometry of peripheral blood samples taken during the study period revealed low pretreatment numbers of absolute $CD3^+CD25^+$ lymphocytes, and this did not change after treatment with denileukin diftitox. However, there was a marginally significant reduction in absolute NK cell and in $CD3^+$ lymphocyte counts 1 to 7 days after treatment, followed by brisk recovery after the third week. The reduction in absolute $CD3^+$ count by 1 to 7 days was statistically significant in patients who eventually achieved a CR ($P = .03$) but was unchanged in nonresponders. Serum or plasma sIL-2R levels were comparable to control post–hematopoietic stem cell transplantation patients without GVHD, and sIL-2R levels remained stable after denileukin diftitox. There was no correlation between sIL-2R level and clinical response.

Summary

Denileukin diftitox can be safely administered in patients after allogeneic hematopoietic stem cell transplantation. A reversible increase in hepatic transaminases is the major DLT, but the incidence (22% at the MTD) is comparable to the 17% reported in patients with cutaneous T-cell lymphoma [32]. Denileukin diftitox seems to have significant clinical activity in steroid-refractory acute GVHD, with complete and overall response rates of 46% and 69%, respectively, at the MTD.

Although the numbers of peripheral $CD3^+$ $CD25^+$ cells were not affected by treatment, absolute numbers of peripheral $CD3^+$ lymphocytes were transiently depleted after treatment with denileukin diftitox, especially in patients who achieved a CR. These results suggest that denileukin diftitox preferentially targets $CD3^+$ T cells that are involved in the GVHD response and that resistance to therapy is associated with the inability of this agent to eliminate these cells in some patients. Because treatment resulted in the elimination of $CD3^+$ T cells that did not express high levels of CD25, these observations suggest that cells expressing other components of the IL-2R may also internalize sufficient denileukin diftitox to result in cell death in vivo. Alternatively, flow cytometry may not be able to detect relatively low-level expression of high-affinity receptors that may still be sufficient to bind and internalize denileukin diftitox.

Despite the encouraging GVHD responses and improved survival among the patients who achieved a CR, the overall survival for this entire cohort was disappointing, and infections remained a common cause of late mortality. Attribution of late infections to denileukin diftitox is difficult in this study because these patients had received many prior immunosuppressive therapies. Future clinical trials investigating denileukin diftitox as primary therapy or in a more viable population of patients with steroid-

refractory disease are needed to confirm the responses reported here and to determine whether this agent improves survival for patients with acute GVHD.

MYCOPHENOLATE MOFETIL

Mycophenolate mofetil ([MMF] CellCept; Roche Laboratories, Indianapolis, IN) is a morpholinoethyl ester of mycophenolic acid (MPA). MPA possesses antibacterial, antifungal, antiviral, antitumor, and immunosuppressive properties. However, to facilitate absorption, it must be administered as the prodrug, MMF. Approximately 95% of the oral dose of MMF is absorbed, and the immunosuppressive activity is evident after de-esterification to MPA in vivo [33]. MPA mediates its immunosuppressive effect by inhibiting inosine monophosphate dehydrogenase, an enzyme that catalyzes the oxidation of inosine monophosphate to xanthine monophosphate, an intermediate metabolite in the synthesis of guanosine triphosphate. Lymphocytes rely on the de novo purine synthesis pathway for the nucleotides necessary for DNA synthesis, whereas other cells can also use the salvage pathway [34-36]. Therefore, MPA depletes the nucleotide pool, resulting in relatively selective suppression of B- and T-cell proliferation compared with myeloid cells. In addition, guanosine triphosphate depletion may prevent recruitment of leukocytes to sites of inflammation by inhibiting the glycosylation of lymphocyte glycoproteins that are involved in intercellular adhesion [37].

Pharmacokinetics of MMF

MMF is usually given orally or intravenously at doses of 2 to 3 g/d for adults. However, therapeutic monitoring has not been formally adopted to optimize immunosuppressive efficacy or to minimize toxicity, despite published reports regarding MMF's pharmacokinetics. MMF is rapidly and completely hydrolyzed to MPA, which is further metabolized by glucuronyl transferase to form a phenolic glucuronide conjugate (MPAG), an inactive metabolite. MPAG is excreted in the bile and urine. Although the liver is the predominant organ for the metabolism of MMF, the gastrointestinal tract and kidney also contribute. MMF is not measurable systemically in plasma after oral administration. The mean half-life of MPA in plasma is 17.9 ± 8.5 hours after oral administration and 16.6 ± 5.8 hours after intravenous administration. Peak levels of MPA occur within 1 to 2 hours of the oral dose, and secondary peaks are usually observed at 6 to 12 hours because of enterohepatic circulation. In volunteers with severe renal impairment, the plasma MPA area under the curve (AUC) was 75% higher than that observed in healthy volunteers. However, this is not predictable, and dose reduction is not necessarily recommended in the presence of renal failure. Less than 1% MMF is excreted as in the urine. In a single-dose study of 18 volunteers with alcoholic cirrhosis, hepatic MPA glucuronidation was relatively unaffected by hepatic parenchymal disease. Dose adjustment has not been recommended for patients with liver failure.

Several studies have addressed the use of therapeutic drug monitoring of MPA in solid organ transplant recipients, although there is limited pharmacokinetic information in stem cell allograft recipients. After kidney or cardiac transplantation, there was a significant association between plasma concentrations and the development of biopsy-proven rejection [38-40]. The importance of therapeutic drug monitoring of MPA is stressed by the fact that in a study of 28 solid organ transplant recipients, Braun et al. [41] found no correlation between the MMF dose administered and MPA levels. A consensus meeting regarding the monitoring of MPA has recommended a therapeutic window for MPA AUC of 30 to 60 μg × h/mL and for trough levels of 1.0 to 3.5 mg/L [42].

Very limited data are available regarding MPA plasma concentrations and pharmacokinetics after hematopoietic stem cell transplantation. In studies of MMF for both prevention and treatment of acute GVHD, MPA plasma concentrations were low with both the oral and intravenous formulations. In one report in which the oral formulation of MMF was used for GVHD prophylaxis, the mean MMF trough level was only 0.28 mg/L [43]. Other studies of GVHD prophylaxis, even with the intravenous formulation of MMF, have confirmed the low plasma concentrations of MPA in the period early after transplantation [44,45]. In a study of acute GVHD treatment, MPA AUC was low (at 17.8 μg × h/mL) with standard doses of the oral formulation of MMF [46]. Secondary peak plasma concentrations are much lower after hematopoietic cell transplantation, effectively shortening the half-life of the drug. The enterohepatic circulation of MPA may be significantly reduced by gut toxicity from the myeloablative conditioning, by the presence of gastrointestinal GVHD, or by antibiotics that reduce the gut flora. Thus, daily MMF doses after stem cell transplantation may need to be higher than the doses used for solid organ transplantation. It may be more effective to increase the daily dose of MMF by shortening the dosing intervals rather than by increasing the dose and maintaining the twice-daily administration. Further studies of MMF in the management of both acute and chronic GVHD should consider the routine monitoring of MPA plasma concentrations.

The pharmacokinetic effects of concurrent administration of calcineurin inhibitors on the disposition of MMF have been evaluated in stable renal allograft recipients [47]. When compared with a cohort of

Table 2. *Treatment of Acute GVHD with MMF: Phase II Studies*

Study	No. Patients	MMF (g/d)	Primary/Secondary	Response
Basara [53,54]	36	2	Primary	26/36 (72%); controls 5/14 (36%)
Nash [46]	19	2–3	Secondary	8/19 (42%)
Abhyankar [56]	7	2	Secondary	2/7 (29%)
Baudard [55]	6	2	Secondary	4/6 (67%)

patients receiving cyclosporine (CSP) and MMF, patients treated with tacrolimus and MMF had significantly higher trough levels and AUC of MPA. These early results seemed to suggest that tacrolimus administration resulted in higher MPA levels when compared with CSP for a given dose of MMF. More recent data suggested that CSP may decrease MPA levels and that tacrolimus does not interact with MPA. In one study, patients receiving CSP, MMF, and prednisone were randomized to remain on all 3 drugs, discontinue prednisone, or discontinue CSP [48]. The MPA levels remained unchanged in the first 2 groups but increased significantly upon discontinuation of CSP in the third group. In another study, the trough levels of MPA in the tacrolimus group were similar to those of patients treated with MMF only. These results have been corroborated in a rat kidney transplant model [49]. It was hypothesized that coadministration of CSP with MMF may decrease trough MPA levels by inhibiting the secretion of MPAG into the biliary and gastrointestinal tract, which decreases the availability of MPA for reabsorption. This significantly reduces the secondary plasma peak and the total AUC of MPA. MMF doses may need to be adjusted if patients have their calcineurin inhibitor changed, though clinical data supporting the benefits of this dosing strategy have not been reported.

MMF in Acute GVHD

MMF is a useful adjunct to calcineurin inhibitors in preventing and treating organ graft rejection [50]. Experience with MMF after hematopoietic stem cell transplantation is more limited. Preclinical studies in a murine GVHD model did not confirm the effectiveness of MMF in the control of GVHD [51]. Studies in a canine model showed that MMF alone was not very effective in preventing GVHD, but in combination with CSP it was comparable to or better than methotrexate and CSP [52]. In a preclinical model of nonmyeloablative transplantation, MMF and CSP after transplantation enhanced engraftment. Phase II clinical studies have been conducted on the combination of MMF with a calcineurin inhibitor for GVHD prevention and treatment [43,44,46,53-55]. Four groups have reported their experience with the treatment of acute GVHD after hematopoietic cell transplantation (Table 2).

Basara et al. [54] reported their experience with primary treatment of grade I to IV acute GVHD in 36 patients after hematopoietic cell transplantation with myeloablative conditioning. GVHD prophylaxis consisted of CSP, methotrexate, and prednisolone. MMF was started at a dose of 250 mg orally 4 times daily, and after a week the dose was increased to 2 g daily. Treatment with MMF for acute GVHD was initiated between days 15 and 82. Of the 36 patients with acute GVHD, 26 (72%) had an overall grade improvement. The best response was observed in the skin. Only 4 of 8 patients with gastrointestinal GVHD responded. This study was difficult to evaluate because the criteria for assessing the response of acute GVHD were not well defined. A total of 48 patients in this study with either acute or chronic GVHD were evaluated for drug-related adverse events. Leukopenia was noted in 9 patients, but it did not necessitate discontinuation of MMF. Adverse gastrointestinal events occurred in 6 of 48 patients. The estimated 5-year survival was 37%.

Nash et al. [46] reported their experience with MMF as a treatment for steroid-refractory grade II to IV acute GVHD. Nineteen patients who had all received myeloablative conditioning were enrolled in the study. In 18 of the 19 patients, the GVHD prophylaxis was CSP and methotrexate. All patients received the oral formulation of MMF at 1 g twice daily. A response was observed in 8 (42%) patients (6 CR and 2 PR). MMF was discontinued in 3 patients because of neutropenia and in 1 patient because of persistent nausea. It was stopped in 2 other patients for GVHD progression. In pharmacokinetic studies, the MPA AUC was less in patients with acute GVHD than in a cohort of patients from the same center with chronic GVHD. Overall survival at 2 years was 16%. In 2 other small reports, responses to therapy were observed in 6 (46%) of 13 patients [55,56].

Summary

MMF is an interesting agent for use in GVHD prophylaxis and therapy. It has the advantage of a good therapeutic index, and it is a relatively selective inhibitor of T-cell metabolism. Although the literature is sparse, MMF seems to have significant activity in phase II studies of primary and secondary therapy of acute GVHD. Treatment of acute GVHD with-

MMF may achieve better control of GVHD with less use of corticosteroids. Adverse effects have primarily involved the gastrointestinal tract and hematopoietic system but have usually not been severe and are reversible with discontinuation of the drug. Plasma concentrations are low in general after hematopoietic cell transplantation compared with solid organ transplantation. This suggests that improved control of GVHD may be achieved by optimizing the dose on the basis of therapeutic drug monitoring.

PENTOSTATIN

Pentostatin (Nipent; Supergen, San Ramon, CA) is a nucleoside analog that is a potent, irreversible inhibitor of adenosine deaminase [57]. This mechanism of action results in molecular and cellular effects similar to congenital deficiency of adenosine deaminase [58], a form of severe combined immune deficiency with marked T lymphopenia [59]. Inhibition of adenosine deaminase blocks the metabolism of 2′-deoxyadenosine, and in lymphocytes, the high ratio of deoxycytidine kinase to 5-nucleotidase favors the formation of 2′-deoxyadenosine 5′-triphosphate from 2′-deoxyadenosine [57]. The accumulation of 2′-deoxyadenosine 5′-triphosphate in lymphocytes slows cell growth and causes apoptosis, thereby reducing the number of T cells and NK cells [60-62]. By inhibiting both the production of IL-2 by T cells and their response to IL-2 [63], pentostatin diminishes T-cell function [58] and inhibits antibody-dependent cellular cytotoxicity and NK cytolytic activity [64]. Moreover, pentostatin reduces the production of TNF-α [65,66] by lipopolysaccharide-treated monocytes, an important component in pathophysiologic models of acute and chronic GVHD [67,68]. In comparison with the other available purine nucleoside analogs, pentostatin has relatively mild myeloid toxicity [69]; however, the persistence of lymphopenia for months after treatment [60,61] may be advantageous in preventing recurrent flares of GVHD and perhaps in preventing subsequent chronic GVHD.

Preclinical data indicate that purine nucleoside analogs may have a potent effect on GVHD. Both pentostatin and fludarabine have prevented GVHD and improved survival in mouse models of allogeneic bone marrow transplantation [70,71].

Pharmacokinetics of Pentostatin

Pharmacokinetic analysis was performed after each dose for the first 13 patients. The observed data fit a model of biexponential decay well, in accordance with previous pharmacokinetic studies [72-74]. Although there was considerable interpatient variation, such that the pentostatin exposure ranges overlapped between dose levels, there was a strong association between the assigned dose level and AUC ($r^2 = 0.51$; $P < .05$). Two patients had pharmacokinetic monitoring after dose adjustments for renal insufficiency according to protocol, and the exposure levels they achieved indicated that these dose adjustments were appropriate. In addition, 1 patient with liver failure had pharmacokinetic monitoring that demonstrated an unexpectedly large exposure to pentostatin. This exposure may have resulted from the distribution of pentostatin into third-space fluid, which would then act as a reservoir for prolonged exposure. A pharmacodynamic effect was observed on lymphocyte counts. Although lymphopenia was universally observed, higher pentostatin AUC was associated with lower lymphocyte counts 7 days after starting treatment with pentostatin ($r^2 = 0.33$; $P = .14$). The sample size was insufficient to assess a relationship between pentostatin exposure and either clinical responses or toxicities, but responses have occurred at exposures that ranged from AUCs of 1000 to 2600 ng × h/mL.

Pentostatin in Acute GVHD

Vogelsang et al. conducted a phase I dose-escalation study to find the MTD of pentostatin with clinical activity in patients with steroid-refractory acute GVHD [75]. A total of 24 patients were enrolled, and 23 were evaluable. Their ages ranged from 6 months to 63 years (median, 43 years). All had biopsy-proven grade II to IV acute GVHD that was refractory to methylprednisolone (MP) at a dose of at least 2 mg/kg/d. Myeloid engraftment with an absolute neutrophil count of >1000/μL was required for entry onto the study. Patients were to be assigned in cohorts of 5 to dose levels of 1, 2, 3, or 4 mg/m^2/d intravenously for 3 days. Toxicities were scored according to the National Cancer Institute common toxicity criteria version 2.0, as modified for hematopoietic stem cell transplantation studies, and grade 3 or 4 toxicities were considered dose limiting if they were attributable to pentostatin. The dose of pentostatin was modified for renal insufficiency on the basis of the observed or estimated creatinine clearance, as follows: for creatinine clearance 30 to 50 mL/min/1.73 m^2, 50% of the dose was given; at <30 mL/min/1.73 m^2, the dose was held constant. Steroids were tapered by 0.5 mg/m^2/d every fourth day either until the patient reached physiologic replacement doses or, for patients whose GVHD prophylaxis included steroids, until the patient reached the planned prophylactic dose. All patients had experienced failure of primary therapy of GVHD with steroids. Nine patients had experienced failure of at least 1 additional line of therapy for acute GVHD.

Table 3. *Clinical Outcomes of Patients with Steroid-Refractory Acute GVHD Treated at Various Dose Levels of Pentostatin*

Dose Level*	Response				Survival	GVHD-Specific Mortality
	CR	PR	MR	NR		
1	5 (50%)	1 (10%)	2 (20%)	2 (20%)	2 (20%)	3 (30%)
1.5	4 (80%)			1 (20%)	2 (40%)	3 (60%)
2	5 (83%)	1 (17%)			2 (33%)	1 (17%)
3	0	1 (100%)			0 (0%)	0 (0%)

Deaths were attributed to refractory acute GVHD, refractory chronic GVHD, or bronchiolitis obliterans.
MR indicates mixed response; NR, no response.
*Dose in mg/m^2/d, intravenously, for 3 days.

A single course of 3 days of pentostatin was specified by the protocol, but patients who responded to pentostatin and subsequently experienced disease flares were treated again at the same dose level. Pentostatin was generally well tolerated. Lymphopenia was universally observed and was a desired effect of treatment. One patient each developed grade 3 thrombocytopenia (at 1 mg/m^2/d), grade 1 neutropenia (at 1.5 mg/m^2/d), and grade 1 transaminase increases (at 1.5 mg/m^2/d). The DLT was believed to be late infections at a dose of 2 mg/m^2/d for 3 days. A major challenge of this study was the attribution of infectious complications, because this patient population has an intrinsic susceptibility to infection. Nevertheless, the pattern of infections, including fatal infections with adenovirus, human herpesvirus 6 pneumonia, cytomegalovirus, and sepsis, seemed to be attributable to the cellular immune deficiency associated with pentostatin. The dosage of pentostatin was therefore de-escalated. After accrual of an additional 5 patients at 1 mg/m^2/d, an intermediate dose level of 1.5 mg/m^2/d was established and selected as the MTD (Table 3).

Responses were evaluated at least weekly until day 28 on study. One patient died of preexisting liver failure on day 5 on study; this was deemed unrelated to the study drug. The remaining 22 patients were evaluable for response. Fourteen patients achieved a CR (64%), 3 had a PR (7%), 2 had a mixed response, 3 maintained stable disease, and 1 developed progressive disease. By organ system, the response rate for skin involvement was 13 CR (81%), 1 PR (6%), and 2 no response; for gut, it was 11 CR (79%), 2 PR (14%), and 1 no response; and for liver, it was 6 CR (55%), 1 PR (9%), and 4 no response. Six of the patients who initially responded had disease flares with acute GVHD and were re-treated. All of these patients responded to treatment at the same dose of pentostatin.

The median survival remained poor—91 days (range, 5-1239 days) from study entry. However, an encouraging finding was that long-term survival reached a plateau at 26%, at a median follow-up of 340 days (range, 180-1059 days). The major cause of death was infection. Four patients died of refractory acute GVHD, 1 of refractory chronic GVHD, and 2 of bronchiolitis obliterans. Only 2 patients had relapses of the underlying disease. These patients were treated with donor lymphocyte infusions and succumbed to infections afterward.

Summary

Pentostatin has encouraging activity in steroid-refractory acute GVHD, and most patients achieved a CR and had no evidence of active GVHD within 4 weeks of treatment with a single 3-day course of pentostatin. However, a substantial minority of patients experienced disease flares with acute GVHD and required a second course of therapy. Mortality remained high, primarily because of infections, despite aggressive protocol-mandated tapering of steroids in the patients with steroid-refractory disease. Pentostatin exposure, as measured by AUC, was significantly associated with the assigned dose level, and although the dose adjustment for renal insufficiency was appropriate, it is also important to consider dose reduction in the setting of hepatic failure, presumably due to the presence of third-space fluid. The recommended dose for phase II investigation is 1.5 mg/m^2/d intravenously for 3 days, with a second course to be administered in 3 weeks, except in patients who have significant infections or who remain severely lymphopenic. Additional pharmacokinetic data would be of great value in a phase II trials to clarify the relationship between exposure and clinical outcomes. It is possible that exposure-based or glomerular filtration rate–based dosing would produce more consistent outcomes than dosing on the basis of body-surface area. Ultimately, using this therapy earlier in the course of GVHD is likely to be necessary to avoid the mortality associated with steroid-refractory GVHD.

INFLIXIMAB

The rationale for the use of infliximab in the therapy of GVHD differs from that for the agents described previously. Because inflammatory cytokines

are important mediators of acute GVHD [2], several investigators have studied known and putative inhibitors of TNF-α and of IL-1. Both the IL-1 receptor antagonist anakinra (Kineret; Amgen, Thousand Oaks, CA) and the sIL-1 receptor had demonstrable activity (50%-60% response rate) in steroid-refractory GVHD [76,77]. Subsequent studies showing that the IL-1 receptor antagonist was ineffective as GVHD prophylaxis dampened enthusiasm for further trials in acute GVHD. However, this drug may deserve a second look as therapy alone or in combination with other cytokine inhibitors.

High levels of TNF-α have been observed in GVHD in bone marrow transplant recipients [78,79]. TNF-α is mainly produced by monocytes and macrophages and secondarily by T lymphocytes and NK cells [80-82]. The cellular effects of TNF-α are mediated through the induction of apoptosis in target tissues, but the cytokine also has profound effects on the immune response by inducing nuclear factor-κB, nitric oxide synthetase, adhesion molecules (eg, intercellular adhesion molecule-1), IL-2Rα, major histocompatibility complex class I and II, and secreted proteins such as IL-1, IL-6, IL-8, IL-12, IFN-β, granulocyte-macrophage colony-stimulating factor, platelet-derived growth factor, and urokinase-type plasminogen activator. In addition, there is activation of macrophages, dendritic cells, neutrophils, eosinophils, B cells, and T cells and facilitation of T-lymphocyte lysis [2,83].

Multiple inhibitors of TNF-α activity have been described, including steroids [84], pentoxifylline [85], transforming growth factor-β [86], and IL-4 [87]. Clinical trials of murine monoclonal antibodies to TNF-α have shown transient improvement in acute GVHD [83,88,89]; however, limitations in the use of murine monoclonal antibodies have prevented large-scale definitive trials. A humanized monoclonal antibody to TNF-α, such as infliximab, might benefit from better pharmacodynamics.

Infliximab (Remicade; Centocor, Malvern, PA) is an immunoglobulin G1 murine/human chimeric monoclonal antibody composed of human constant and murine variable regions that binds both the soluble subunit and the membrane-bound precursor of TNF-α [90]. Infliximab inhibits a broad range of biological activities of TNF-α by blocking its interaction with its receptors, and it may also cause lysis of cells that produce TNF-α [90]. The drug has been used with success for the treatment of a range of autoimmune and inflammatory diseases, including Crohn disease, rheumatoid arthritis, psoriasis, and spondyloarthropathies [91-97]. The median terminal half-life of infliximab ranges from 8 to 10 days, and it is distributed primarily within the vascular compartment.

Infliximab in Acute GVHD

Three studies involving a total of 14 patients with acute GVHD treated with infliximab have been reported [88,98,99]. All patients had advanced steroid-resistant acute GVHD and received infliximab at 10 mg/kg/wk for a median of 4 doses. Significant responses were seen in several patients with severe gastrointestinal disease, and the drug was not apparently associated with any major immediate toxicity.

Couriel et al. [83] conducted a retrospective analysis of 21 patients with steroid-resistant acute GVHD who received infliximab added as a single agent to tacrolimus and corticosteroids for the initial treatment of steroid-resistant acute GVHD. Patients who developed acute GVHD continued on tacrolimus, maintaining blood levels between 5 and 15 ng/mL, and received MP 2 mg/kg/d in divided doses. Patients whose GVHD was steroid resistant received infliximab at 10 mg/kg once weekly for 4 doses (median, 4; range, 2-9); additional doses were allowed for PRs. Tacrolimus was maintained throughout therapy, and MP was tapered from the initial dose of 2 mg/kg/d as tolerated when significant improvement was achieved (at least a PR). Responses were assessed for each involved organ. CRs and PRs were assessed 7 days after the initiation of infliximab. Patients were considered nonresponders if the GVHD had not improved or progressed 7 days after the initiation of infliximab for skin GVHD or 72 hours after its initiation for visceral GVHD. Responses were observed in 14 patients (67%), and 13 of these patients (62%) had a CR. Five patients (24%) did not respond, and 2 (10%) had progression of their GVHD.

The best responses were seen in patients with intestinal GVHD, with 8 CRs and 1 PR among 12 patients. Of 10 patients with cutaneous acute GVHD, 6 had a CR, and 1 had a PR. Of 4 patients with liver involvement, 1 had CR, and 3 did not respond. Age, sex, HLA compatibility (related versus unrelated transplant), and overall grade (grade II versus III/IV) were evaluated as prognostic factors for response to infliximab. None of these factors reached statistical significance. Among patients who responded to infliximab with a CR or a PR, chronic GVHD developed in 93%. There were no infusion, allergic, or other toxic reactions to infliximab.

The duration of response was measured from the time of first infliximab treatment to the day of disease progression or initiation of additional immunotherapy. Response duration exceeded 14 days in all 14 patients with a CR or a PR and exceeded 30 days in 11 patients. Ten of the patients who had a CR after infliximab did not require any further immunosuppressive therapy. The remaining 3 patients subsequently required salvage immunosuppression after brief remissions.

Table 4. *Infections after Infliximab Therapy*

Type of Infection	Patients, n (%)	Positive Cultures, n (%)
Bacterial	17 (81)	40 (49)
Gram-positive	11	32
Gram-negative	5	8
Others	4	5
Fungal	10 (48)	18 (22)
Aspergillus sp	6	7
Candida glabrata	5	5
Candida sp	4	6
Viral	14 (67)	24 (29)
Cytomegalovirus	11	16
Respiratory viruses	5	5
Others	3	3
Total	21 (100)	82 (100)

Table 5. *Causes of Death after Primary Therapy for Acute GVHD with Corticosteroid with or without Infliximab*

Variable	Methylprednisolone	Methylprednisolone + Infliximab
n	28	30
Complete remission	18 (64%)	19 (63%)
Cause of death		
GVHD	8	8
Relapse	8	5
Infection	1	1
Multiorgan failure	0	3
Other	1	2

Infections are summarized in Table 4. These data emphasize that although the salvage therapy with infliximab seemed effective, the risk of infection was substantial. Another retrospective analysis of invasive fungal infection in patients receiving salvage therapy for steroid-resistant GVHD observed a hazard ratio for infliximab exposure of 13.6 ($P = .004$) [100]. Eight patients remained alive at a median follow-up of 21 months (range, 11-31 months). Thirteen patients (62%) died, and all of the 8 surviving patients have chronic GVHD. The Kaplan-Meier estimate of overall survival for all patients from the time of transplantation was 38%. The median survival since transplantation was 8.7 months (95% confidence interval, 0.30 to 0.71). Acute (n = 1) and chronic (n = 8) GVHD were the main causes of death (69%), followed by recurrence of malignancy (n = 3; 23%) [101].

The encouraging response rate in such a poor-risk patient population led to a phase III study of up-front treatment of acute GVHD in hopes of better response and survival with treating GVHD at an earlier stage. Fifty-eight patients with untreated acute GVHD were randomized to receive MP (n = 28) or MP plus infliximab (n = 30) in an open-label trial. Sex, age, organ involvement, and severity of GVHD were similar in both arms (data not shown). Infliximab was well tolerated, and no acute or infusion-related reactions were noted in the steroid-refractory or phase III experience. Overall response (CR/PR) rates were similar in both the MP (63%; n= 15) and the MP plus infliximab (66%; n= 20) groups, with no differences in response by organ. The death rate was similar in

Table 6. *Summary of Activity of Available Agents in Steroid-Resistant Acute GVHD*

Agent	Mechanism	Toxicity	Efficacy			
			n	CR	PR	Overall
Antithymocyte globulin	Complement fixation, ADCC	Infection, EBV lymphoproliferative disorder	—	—	—	19%-56%
Daclizumab	Complement fixation, ADCC of IL-2R–expressing cells	Infection, EBV lymphoproliferative disorder	43	37%	14%	51%
Denileukin diftitox	Intoxication of cells expressing IL-2 receptor	Infection, edema, transaminitis	24	50%	21%	71%
Mycophenolate mofetil	Inhibition of IMPDH; relatively selective for T cells	Infection, nausea, diarrhea	68			59%
Pentostatin	Inhibition of ADA	Infection, myelosuppression	22	64%	14%	73%
Infliximab	Binding of TNF-α	Infection	21	62%	5%	67%

ADCC indicates antibody-dependent cellular cytotoxicity; IMPDH, inosine monophosphate dehydrogenase; ADA, adenosine deaminase; EBV, Epstein-Barr virus.

both study arms as well, and the main cause was GVHD followed by relapse of the underlying malignancy (Table 5).

Summary

Despite initial encouraging results, preliminary results showed no significant differences in response between the MP and MP plus infliximab groups. In the M.D. Anderson phase III experience, the incidence of infection and causative organisms were similar in both groups, although an increased risk of fungal infections has been recently reported, and cautious use of this agent should probably include antifungal prophylaxis [100]. Despite encouraging response rates in the steroid-refractory setting, treatment of acute GVHD at an earlier stage with infliximab did not prove more effective than steroids alone. Once established, GVHD continues to be the main cause of death.

CONCLUSION

A summary of the activity for available agents in steroid-resistant acute GVHD is presented in Table 6. A number of other immunosuppressive agents are demonstrably active as second-line therapy, but each may further predispose patients to life-threatening infections. For instance, neither CD5 immunotoxins [4,102], nor monoclonal antibodies directed against T cells [17,18,21-23,103-107], nor monoclonal antibodies directed against TNF [83,88,89], nor extracorporeal phototherapy [108,109], nor very-high-dose corticosteroids [110,111] have been demonstrably helpful in improving survival [5,7]. Many patients die of infections whether or not the GVHD has entered remission [100]. Because high-dose corticosteroids are a significant risk factor for infections, diabetes, bone loss, and nutritional compromise, intensification of immunosuppression with nonsteroidal agents to control GVHD along with an accelerated taper of steroids may reduce the risk for infections and overall morbidity. Supportive care of patients with GVHD may involve attention to nutritional support, bone mineral retention and repair, and intensified infection prophylaxis. Because the current prognosis for patients with steroid-resistant GVHD is poor and because the graft-versus-tumor reaction is critical for the therapeutic effect of transplantation, emphasis in clinical studies needs to be on initial control of acute GVHD, thus sparing larger patient groups the extended morbidity and mortality that accompany the often ineffective treatment of advanced and steroid-refractory GVHD.

ACKNOWLEDGMENTS

This work was supported by grant nos. HL070149, CA18029, and HL36444 from the National Institutes of Health (Bethesda, MD).

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