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Pathogenesis and Management of Graft versus Host Disease

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Synopsis

Allogeneic hematopoietic cell transplantation (HCT) is an important therapeutic option for a variety of malignant and nonmalignant conditions. As the number of allogeneic HCT continues to increase, greater attention to improvements in supportive care, infectious prophylaxis, immunosuppressive medications and DNA-based tissue typing are continuously being made. However, graft versus host disease (GVHD) remains the most frequent and serious complication following allogeneic HCT and limits the broader application of this important therapy. Recent advances in the understanding of the pathogenesis of GVHD have led to new approaches to its management, including utilizing its role in preserving the graft versus leukemia effect following allogeneic transplant. Here we review the important elements in the complex immunological interactions involving cytokine networks, chemokine gradients and the direct mediators of cellular cytotoxicity that cause clinical GVHD, and discuss the risk factors and strategies for management of GVHD.

Introduction

Allogeneic hematopoietic cell transplantation (HCT) is an important therapeutic option for a variety of malignant and nonmalignant conditions [1]. The indication for its use has expanded, especially among older patients, over the last several years through novel strategies utilizing donor leukocyte infusions, non-myeloablative conditioning and umbilical cord blood (UCB) transplantation [2]. As the number of allogeneic HCT continues to increase, with more than 20,000 allogeneic transplantations performed annually worldwide, greater attention to improvements in supportive care, infectious prophylaxis, immunosuppressive medications and DNA-based tissue typing are continuously being made. Despite advances, graft versus host disease (GVHD) remains the most frequent and serious complication following allogeneic HCT and limits the broader application of this important

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therapy [3]. GVHD can be considered an exaggerated manifestation of a normal inflammatory mechanism in which donor lymphocytes encounter foreign antigens in a milieu that fosters inflammation. In the context of hematological malignancies, a delicate balance exists between the harmful consequences of GVHD and the beneficial effects incurred when donor lymphocytes attack recipient malignant cells, a process referred to as the graft versus leukemia/tumor (GVL) effect. Given the increasing number of transplant recipients, there will be an increasing population of patients with GVHD. Recent advances in the understanding of the pathogenesis of GVHD have led to new approaches to its management, including utilizing its role in preserving the GVL effect following allogeneic transplant. Here we review the important elements in the complex immunological interactions involving cytokine networks, chemokine gradients and the direct mediators of cellular cytotoxicity that cause clinical GVHD, and discuss the risk factors and strategies for management of GVHD.

ACUTE GVHD

Epidemiology and Risk Factors

In 1966, Billingham formulated three requirements for the development of GVHD: the graft must contain immunologically competent cells; the recipient must express tissue antigens that are not present in the transplant donor; and the recipient must be incapable of mounting an effective response to eliminate the transplanted cells [4]. It is now known that T cells are the immunologically competent cells, and when tissues containing T cells (blood products, bone marrow, and solid organs) are transferred from one person to another who is unable to eliminate those cells, GVHD can develop [5, 6].

Allogeneic HCT is the most common setting for the development of GVHD where recipients receive immunoablative chemotherapy and/or radiation prior to hematopoietic cell infusion containing donor T cells. GVHD ultimately develops when donor T cells respond to recipient tissue antigens secondary to mismatches between major and/or minor histocompatibility antigens between the donor and recipient. The major histocompatibility complex (MHC) contains the genes that encode tissue antigens. In humans, the MHC region lies on the short arm of chromosome 6 and is called the HLA (human leukocyte antigen) region [7]. Class I HLA (A, B, and C) proteins are expressed on almost all nucleated cells of the body at varying densities. Class II (DR, DQ, and DP) proteins are primarily expressed on hematopoietic cells (B cells, dendritic cells, monocytes, and activated T cells), but their expression can be induced on many other cell types following inflammation or injury. High-resolution DNA typing of HLA genes with polymerase chain reaction (PCR)-based techniques have now largely replaced earlier methods. The incidence of GVHD is directly related to HLA disparity [8, 9] and with more HLA-mismatches, the likelihood of developing GVHD increases [10, 11]. Recent data from the National Marrow Donor Program (NMDP) suggests that high-resolution matching for HLA-A, -B, -C, and -DRBI (8/8 match) maximizes posttransplant survival [12, 13].

Despite HLA identity between a patient and donor, the incidence of acute GVHD ranges from 26% to 32% in recipients of sibling donor grafts, and 42% to 52% in recipients of unrelated donor grafts (CIBMTR Progress Report January – December 2008). The incidence

is likely related to genetic differences that lie outside the HLA loci, or “minor” histocompatibility antigens (HA), which are immunogenic peptides derived from polymorphic proteins presented on the cell surface by MHC molecules [14]. Some minor HAs, such as HY and HA-3, are expressed on all tissues and are targets for both GVHD and GVL, while other minor HAs, such as HA-1 and HA-2, are expressed abundantly on hematopoietic cells (including leukemic cells) and may induce a greater GVL effect with less GVHD [14, 15]. However, the precise elucidation of the majority of human minor antigens remains to be accomplished [14, 16].

The impact of donor and recipient polymorphisms in cytokine genes with critical roles in the classical ‘cytokine storm’ of GVHD has been examined as a risk factor for GVHD [17]. A variety of polymorphic genes, including tumor necrosis factor (TNF)- α , interleukin 10 (IL-10), and interferon- γ (IFN γ) variants, have been associated with GVHD, although not always [18–20]. At present, there is no unequivocal evidence that polymorphic genes for cytokines or other proteins involved in innate immunity [21–24] sufficiently influence GVHD and transplant outcome to change clinical practice. Nonetheless, future strategies to identify the best possible transplant donor will likely incorporate both HLA and non-HLA genetic factors.

In addition to genetic factors, other risk factors which have been associated with the development of GVHD include older donor and recipient age [25–28], multiparous female donor [28, 29], advanced malignant condition at transplantation [9, 29], donor type [28], and donor hematopoietic cell source [30–32]. Over the last decade, there has been a shift in clinical practice from the use of intraoperative harvested bone marrow (BM) to G-CSF mobilized peripheral blood stem cells (PBSC) as the donor hematopoietic cell source. However, definitive data demonstrating long-term advantages of PBSC rather than BM are lacking. One meta-analysis found that both acute and chronic GVHD are more common following peripheral blood stem cell transplant (PBSCT) compared with bone marrow transplant (BMT) and indicated a trend towards decreased relapse rate following PBSCT [31]. The relative risk (RR) for acute GVHD after PBSCT was 1.16 (95% CI, 1.04 to 1.28) compared with BMT; the RR for chronic GVHD after PBSCT was 1.53 (95% CI, 1.25 to 2.05); and the RR for relapse after PBSCT was 0.81 (95% CI, 0.62 to 1.05). Thus, the survival benefit of PBSC versus BM remains in question. A large prospective, randomized, multicenter clinical trial of PBSC versus BM in unrelated donor transplantation conducted through the Bone Marrow Transplant Clinical Trials Network has recently finished accrual.

For those without a suitable HLA-matched donor, umbilical cord blood (UCB) has become an alternative to bone marrow or peripheral blood stem cells [33–36]. The incidence and severity of acute GVHD appears to be lower following UCB transplant than after HLA-matched marrow unrelated donor transplant, despite HLA disparities between the donor and recipient [37, 38]. In an effort to meet the minimum cell dose required to ensure reliable engraftment, the simultaneous transplantation of two partially HLA-matched UCB units has been studied [39]. A recent report comparing transplantation with two partially HLA-matched UCB units versus a single unit demonstrated an increased incidence and earlier presentation of acute GVHD associated with the double UCB graft [40].

Prevention

Prevention of acute GVHD is an integral component to the management of patients undergoing allogeneic HCT. The primary strategy employed is in the use of pharmacologic GVHD prophylaxis. The most widely used GVHD prophylaxis following full intensity conditioning includes a combination of a calcineurin inhibitor (e.g. cyclosporine, tacrolimus) with methotrexate (MTX). This standard regimen was initially described in 1986 [41] and since then several clinical trials have shown the superiority of this combination in reducing the incidence of GVHD and improving survival compared to either agent alone [42–45]. Calcineurin inhibitors, cyclosporine and tacrolimus, impede the function of the cytoplasmic enzyme, calcineurin, which is critical in the activation of T cells. The most common side effects include hypomagnesemia, hyperkalemia, hypertension, and nephrotoxicity [46]. Large randomized studies comparing tacrolimus-MTX to cyclosporine-MTX have demonstrated a reduced incidence of grade II-IV acute GVHD with tacrolimus, but no overall survival advantage [43, 46].

Recently, sirolimus, a widely used immunosuppressant in solid organ transplantation [47], has become attractive to use as a GVHD prophylactic agent because of the non-overlapping toxicities with calcineurin inhibitors and the different mechanism of action. Sirolimus binds uniquely to FK binding protein 12 (FKBP12) and then complexes with mTOR (mammalian Target of Rapamycin) [48]. Several studies have shown that the combination of sirolimus and tacrolimus has resulted in rapid engraftment, low incidence of acute GVHD, reduced transplant-related toxicity, and improved survival [49]. A prospective, randomized, multicenter trial is currently being conducted through the BMT CTN (protocol 0402) comparing sirolimus-tacrolimus versus tacrolimus-MTX following HLA-matched, related donor peripheral blood stem cell transplantation.

A commonly used GVHD prophylaxis following reduced-intensity conditioning includes a combination of a calcineurin inhibitor (e.g. cyclosporine, tacrolimus) with mycophenolate mofetil (MMF) instead of MTX. MMF, the prodrug of mycophenolic acid, selectively inhibits inosine monophosphate dehydrogenase, an enzyme critical to the de novo synthesis of guanosine nucleotide, which is needed for proliferation of T cells. In a prospective randomized trial, patients who received MMF as part of GVHD prophylaxis experienced significantly less severe mucositis and more rapid neutrophil engraftment than those who received MTX [50]. Although the optimal prophylaxis regimen following reduced-intensity HCT is not well established, mycophenolate mofetil (MMF) has been shown to be safe in this context [51–55]. MMF is also often preferred to MTX in UCB transplants due to its advantageous toxicity profile with respect to neutropenia and mucositis.

Many centers have previously attempted to decrease the risk of GVHD by ex vivo T cell depletion. Despite significant reductions in the incidence and severity of GVHD, T cell depletion has not achieved wide acceptance because of high rates of graft rejection, life-threatening infections and leukemia relapse [56–58]. In vivo T cell depletion has also been widely studied utilizing alemtuzumab, a monoclonal antibody specific for CD52 antigen expressed abundantly on the surface of normal and malignant lymphocytes [59, 60], or anti-thymocyte globulin (ATG), a polyclonal antibody mixture of either horse or rabbit origin directed against multiple epitopes of human T cells [61]. These approaches are associated

with significant reduction in acute GVHD, but at the cost of impaired immune reconstitution and increased risk of leukemia relapse. Thus, the focus of most prevention strategies remains pharmacological manipulation of T cells following transplant.

Pathophysiology

Acute GVHD is mediated by donor lymphocytes infused into the recipient where they encounter profoundly damaged tissues from the effects of the underlying disease, prior infections, and the transplant conditioning regimen. The allogeneic donor cells encounter a foreign environment that has been altered to promote the activation and proliferation of inflammatory cells. Thus, acute GVHD reflects an exaggerated response of the normal inflammatory mechanisms that involves donor T cells as well as multiple innate and adaptive cells and mediators. Three sequential phases can be conceptualized to illustrate the complex cellular interactions and inflammatory cascades that ultimately evolve to acute GVHD: (1) activation of antigen presenting cells (APCs); (2) donor T cell activation, proliferation, differentiation and migration; and (3) target tissue destruction [62].

Phase 1: Activation of APCs

In the first phase, APCs are activated by the underlying disease and the HCT conditioning regimen [63]. Both animal models [63, 64] and clinical HCT [65] have supported the observation that increased risk of GVHD is associated with intensive conditioning regimens which contribute to extensive tissue injury and subsequent release of inflammatory cytokines. Damage to host tissues leads to the secretion of proinflammatory cytokines, such as TNF- α and IL-1, and chemokines, such as CCL2-5 and CXCL9-11, thereby producing increased expression of adhesion molecules, MHC antigens and costimulatory molecules on host APCs. For example, elevation of plasma TNF- α receptor 1 levels, a surrogate marker for TNF- α , at one week after HCT strongly correlates with the later development of GVHD [66]. Systemic translocation of immuno-stimulatory microbial products, such as lipopolysaccharide (LPS), as a result of conditioning regimen induced damage to the gastrointestinal (GI) tract, enhances the activation of host APCs [67, 68]. The initial site of interaction between activated APCs and donor T cells is likely the secondary lymphoid tissue in the GI tract [69]. Different distinct subsets of APCs, including host and donor type APCs [68, 70, 71], dendritic cells [72, 73], Langerhans cells [74], and monocytes/macrophages [75], have been implicated in this phase. However, the relative contributions of these various APCs remain to be elucidated. The intensity of the conditioning regimen and the degree of tissue injury appear to be associated with the risk of GVHD. Reduced intensity conditioning regimens have thus become more widely employed in an effort to reduce acute GVHD by decreasing the damage to host tissues [65, 76].

Phase 2: Donor T Cell Activation

Donor T cell activation, proliferation, differentiation and migration in response to primed APCs occurs during the second phase of acute GVHD. The T cell receptors (TCR) of donor T cells recognize alloantigens on both host and donor type APCs that are present in secondary lymphoid organs [77, 78]. During direct presentation, donor T cells recognize either the peptide bound to host MHC molecules, or the foreign MHC molecules themselves

[79]. During indirect presentation, donor T cells respond to the peptides generated by degradation of the host MHC molecules that are presented on donor-derived MHC [80].

Following antigen recognition, signaling through the TCR induces a conformational change in adhesion molecules, resulting in high affinity binding to the APC [81]. The complex interaction between T cell co-stimulatory molecules and their ligands on APCs facilitates full T cell activation. A large number of T cell costimulatory molecules display both unique and overlapping functions [82]. Receptors of the B7 family and the TNF family play especially critical roles in GVHD, and are known to deliver both positive and negative signals to T cells [83]. Blockade of costimulatory and inhibitory pathways can reduce acute GVHD in murine models, but this approach has not yet been tested in clinical trials [2].

Murine studies have shown that control of alloreactive responses responsible for GVHD depends at least in part on CD4⁺ CD25⁺ regulatory T cells. Studies in mice suggest that regulatory T cells added to donor grafts can prevent or delay GVHD [84]. However, the role of regulatory T cells in clinical allogeneic HCT has not been well established in part due to the lack of clear identification of human regulatory T cell phenotype. In contrast to murine studies, more severe acute GVHD developed clinically when donor grafts contained larger numbers of donor CD4⁺ CD25⁺ T cells [85]. One recent study found that HCT recipients with higher absolute numbers of FOXP3⁺ CD4⁺ T cells were associated with a reduced risk of developing GVHD [86]. However, FOXP3 expression in humans is not specific for regulatory T cell phenotype [87], and improved techniques to identify and expand regulatory T cells are required for its wider application in clinical BMT.

Several intracellular biochemical pathways are rapidly amplified following T cell activation. Activated T cells secrete cytokines that are generally classified as Th1 (IFN- γ , IL-2 and TNF- α) or Th2 (IL-4, IL-5, IL-10 and IL-13) and Th17. Although Th1 cytokines induce GVHD efficiently, the balance of Th1 and Th2 cytokines is important in the immunopathogenesis of GVHD, but remains incompletely understood [88]. TNF- α , already discussed as an inducer of APC activation in phase I, functions to amplify T cell activation and proliferation in this second phase of GVHD. IL-2 production is also integral in this early development of acute GVHD [89], and remains the principal target of many current clinical therapeutic and prophylactic approaches to GVHD, such as cyclosporine, tacrolimus and monoclonal antibodies against IL-2 and its receptor [44]. However, recent data suggest that regulatory T cell function is dependent on the presence of calcineurin-dependent IL-2, and that interference with IL-2 may possibly antagonize the induction of tolerance [90].

IFN- γ is a cytokine with diverse effects in vivo that can both amplify and suppress acute GVHD. With regards to amplification, IFN- γ increases the expression of molecules such as chemokine receptors, MHC proteins, and adhesion molecules. IFN- γ also sensitizes monocytes and macrophages to stimuli such as LPS, thereby accelerating intracellular cascades in response to these stimuli [88]. IFN- γ may also amplify GVHD by directly damaging target cells in the GI tract and in the skin while conversely inducing nitric oxide-mediated immunosuppression [91]. Interestingly, IFN- γ itself can prevent experimental GVHD by hastening the apoptosis of activated donor T cells [92]. Thus, this complexity makes it a challenging target with respect to therapeutic intervention.

IL-10 can suppress the expression of proinflammatory cytokines, chemokines, and adhesion molecules, as well as antigen-presenting and costimulatory molecules in monocytes/macrophages, neutrophils, and T cells [93]. Recent clinical data suggest that genetic polymorphism in the IL-10 promoter region of the recipient has a significant impact on the risk of developing acute GVHD [94]. Increased IL-10 production by recipient cells stimulated *ex vivo* has been associated with a reduced risk of acute GVHD [95]. Experimental data have also demonstrated that TGF- β , another suppressive cytokine, attenuates acute GVHD, but may lead to chronic GVHD [96]. Thus multiple cytokines are important in GVHD pathogenesis and regulation. Furthermore, the timing and duration of cytokine expression may be a critical factor determining the induction of the GVH reaction, and cytokine dysregulation could potentially contribute to the severity of acute GVHD [62].

Phase 3: Cellular and Inflammatory Effector Phase

A complex cascade of cellular and inflammatory mediators occurs during the effector phase of acute GVHD. These mediators synergize to amplify local tissue injury and damage target tissues. As such, the effector phase of GVHD involves aspects of both the innate and adaptive immune response as well as interactions with the proinflammatory cells and cytokines generated during phase 1 and 2.

Cellular Effectors—Cytotoxic T cells (CTLs) are the major cellular effectors of acute GVHD and lyse target cells using principally the Fas/Fas ligand (FasL) and the perforin/granzyme pathways [97]. The Fas-FasL pathway appears to predominate in hepatic GVHD whereas the perforin/granzyme pathways are more important in the GI tract and skin [2]. The “danger” signals generated in phase 1, augmented by the expression of costimulatory molecules in phase 2, induce the production of chemokines. The migration of donor T cells from lymphoid tissues to the target organs where damage occurs is directed by these chemokine gradients and adhesion molecules such as L-selectin and $\alpha_L B_2$ integrin. The up-regulation of chemokines, such as CCL2, CCL3, CCL4, CCL5 and CXCL9, CXCL10, and CXCL11 in GVHD target organs (skin, liver, and gut), play primary roles in this homing process [98]. Furthermore, integrin receptors, which are regulated by chemokines, mediate many adhesive interactions that are critical for successful T cell migration [99].

Inflammatory Effectors—The secretion of inflammatory cytokines, such as TNF- α or IL-1 also results in GVHD target organ injury. The secretion signal may be provided through toll-like receptors (TLRs) by LPS and other microbial products that have leaked through damaged intestinal mucosa by the conditioning regimen in phase 1 [100]. TNF- α is produced by both donor and host cells and is a critical component of GVHD pathophysiology. TNF- α can (1) activate APCs and enhance alloantigen presentation, (2) recruit effector cells to target organs through the induction of inflammatory chemokines, and (3) directly cause tissue damage via apoptosis and necrosis. IL-1 is also involved in the pathogenesis of acute GVHD [17]. Its secretion occurs predominantly in the spleen and skin during the effector phase of experimental GVHD [101]. Increased expression of mononuclear IL-1 mRNA has been associated with the development of GVHD [102]. However, the blockade of IL-1 using recombinant human IL-1 receptor antagonist (IL-1Ra) in patients undergoing HCT did not reduce the risk of GVHD [103].

Clinical Features

The three main organs involved in acute GVHD are the skin, liver, or gastrointestinal (GI) tract. GVHD presents clinically in an acute or chronic form. Historically, the acute and chronic forms were arbitrarily defined based on the timeframe post-transplant (less than or greater than 100 days, respectively) [104]. However, current consensus is that clinical manifestations guide whether the signs and symptoms of GVHD are acute, chronic, or an overlap syndrome (wherein diagnostic or distinctive features of acute and chronic GVHD appear together) [105].

The extent (stage) of involvement of the three principal target organs determines the overall severity (grade) of acute GVHD (Table 1) [106]. The overall grades are classified as I (mild), II (moderate), III (severe) and IV (very severe). A poor prognosis is associated with severe grade III or IV GVHD, with 25% and 5% overall survival, respectively [107].

Skin is generally the first and most commonly affected organ [104]. The presentation of skin involvement generally coincides with donor cell engraftment and is characterized by an erythematous, maculopapular rash that is often pruritic. In severe cases the skin may blister and ulcerate [108]. The pathognomonic histopathologic finding is apoptosis at the base of epidermal rete ridges. Other features include dyskeratosis, exocytosis of lymphocytes, satellite lymphocytes adjacent to dyskeratotic epidermal keratinocytes, and a perivascular lymphocytic infiltration in the dermis [1, 109].

Liver GVHD can be a challenging diagnosis. The initial feature typically includes the development of jaundice or an increase in the alkaline phosphatase and bilirubin; hepatomegaly may be noted. However, it is often difficult to distinguish from other causes of liver dysfunction following allogeneic HCT, such as drug toxicity, veno-occlusive disease, opportunistic (bacterial, viral and fungal) infections, TPN, acalculous cholecystitis, and iron overload, because of the overlapping patterns of clinical history, physical examination, and laboratory and imaging data. Thus, a biopsy is often required to confirm the diagnosis of liver GVHD [110]. The histologic features are endothelialitis, lymphocytic infiltration of the portal areas, pericholangitis, and bile duct destruction [111]. However, the increased risk of bleeding associated with thrombocytopenia in the immediate posttransplant period can preclude obtaining a biopsy. As such, the diagnosis of liver GVHD is often a clinical diagnosis of exclusion.

GI tract involvement of acute GVHD may present as nausea, vomiting, anorexia, diarrhea, and/or abdominal pain. It is a pan-intestinal process with focal lesions of widely varying intensity. Gastric involvement gives rise to postprandial vomiting that is not always preceded by nausea. The diarrhea of GVHD is secretory and may be accompanied by significant GI bleeding as a result of mucosal ulceration, which is a prognostic factor for poor outcome [112]. In advanced disease, diffuse, severe abdominal pain and distension is accompanied by voluminous diarrhea. The histologic features include patchy ulcerations apoptotic bodies in the base of crypts, crypt abscesses, and loss as well as flattening of the epithelium surface [113].

Diagnostic Approach

The diagnosis of acute GVHD is based entirely on clinical criteria that can be confirmed by biopsy of one of the three target organs. Laboratory data and/or imaging studies are also useful tests in the diagnostic approach to GVHD. However, a major challenge with the diagnosis is the absence of laboratory tests that can reliably predict or screen for the condition prior to its onset: establish a diagnosis in real-time; distinguish it from other conditions that present with similar symptoms, such as infection; or stratify patients according to response to therapy. Thus, experimental blood tests with predictive value for GVHD such as a four biomarker panel [114, 115] may ultimately be useful to further identify high-risk groups and their outcomes (Figure 1). These biomarkers could potentially result in immunosuppressive treatment schemas tailored to patients in several risk groups.

Treatment

Despite routine treatment with calcineurin-based prophylaxis, GVHD remains a major complication of allogeneic HCT. The most important predictor of long-term survival in patients with acute GVHD is the primary response to the first-line of treatment [116]. Many centers treat mild skin GVHD (grade I) with topical corticosteroids alone, but for more severe skin GVHD and/or visceral GVHD involvement (grade II or greater), systemic corticosteroids are the mainstay of therapy, typically starting at 1–2 mg/kg/day. Unfortunately, durable responses occur in less than half of patients with grade II to IV GVHD [117], and with increasing severity of the disease, the likelihood of response decreases [104]. Treatment with high-dose steroids often continues for 7 days or more, with a gradual dose-reduction depending on the clinical response. In a recent retrospective analysis, low-dose (1 mg/kg/day) versus high-dose (2 mg/kg/day) prednisone was compared for initial treatment of acute GVHD. In patients with grades I-II GVHD, the non-relapse mortality and overall survival were similar between regimens, with a reduced risk of invasive fungal infections and shorter hospitalizations in the low-dose prednisone group. The number of patients with grades III-IV at onset was too few to draw any significant conclusions.

In addition to topical corticosteroid therapy for skin GVHD, non-absorbed steroid therapy is commonly used in GI GVHD. Oral budesonide in combination with systemic corticosteroids in patients with grade II acute GI GVHD has shown complete responses in 77% of patients compared to 32% of historical controls [118]. In a more recent randomized, placebo-controlled trial of oral beclamethasone for GI GVHD, oral BDP reduced GVHD flares following a prednisone taper and resulted in superior survival at 1 year posttransplant [119]. Intra-arterial administration of steroids for GI and hepatic GVHD has also been attempted in order to deliver steroids directly to the target organ [120].

Prolonged therapy with steroids invariably increases the risk of patients developing muscle weakness and wasting, avascular necrosis, compression fractures, hypertension, hyperglycemia, behavior disturbances, and life-threatening infections. Furthermore, the optimal dose and duration of high-dose steroids, as well as the choice and timing of when to institute second therapy remain unclear [121]. Thus, the management of a patient who develops steroidrefractory acute GVHD is challenging. Generally, steroid-refractoriness is

defined as disease progression or lack of response following 3–7 days of systemic therapy with corticosteroids and a calcineurin inhibitor. Once steroidrefractory GVHD develops, a variety of agents may be used and there is currently no standard approach practiced uniformly by the transplant community.

Table 2 summarizes the various drugs that have been studied in addition to steroids for treatment of acute GVHD. Among the agents discussed, mycophenolate mofetil (MMF), etanercept, denileukin diftitox and pentostatin were most recently studied in a clinical investigation through the BMT CTN (study 0302). In this phase II, randomized trial of 180 patients with newly diagnosed acute GVHD, patients were treated with standard corticosteroids plus one of the four agents. MMF produced the best results with regards to toxicity profile, response, survival, incidence of chronic GVHD, and infections [122]. A randomized phase III study to validate these findings is currently being planned.

Extracorporeal photopheresis (ECP) is increasingly used in the management of acute and chronic GVHD in an effort to minimize corticosteroid exposure. During ECP, the patient's peripheral blood mononuclear cells are exposed to photo-activated 8-methoxypsoralen (8-MOP) and ultraviolet A radiation, which covalently binds and cross-links DNA, and then returned to the patient. Experimental studies in humans and mice suggest ECP induces apoptosis of the mononuclear cells and increases the number of regulatory T cells [123, 124]. A phase II study of steroidrefractory GVHD showed complete response rates of 60% with overall survival of 59% at 4 years [125]. These data are similar to other studies which have demonstrated complete response rates between 52% and 83% [126–128].

Mesenchymal stromal cell infusion is another non-pharmacologic approach, but little is known about its mechanism of action. In vitro, mesenchymal stromal cells have various effects on immune cells, including T-cells, antigenpresenting cells, natural-killer cells, and B-cells. In phase I and II trials, HLA-identical mesenchymal stromal cells expanded ex vivo have been infused to promote hematopoietic recovery after autologous and allogeneic HCT [129, 130]. In a phase II study, mesenchymal stem cells were used for the treatment of corticosteroid-resistant, severe, acute GVHD [130]. Thirty-nine of 55 patients with corticosteroid-resistant, severe, acute GVHD responded to treatment with mesenchymal stem cells. In those who achieved a complete response, survival was significantly higher and transplant-related mortality was lower compared to those with partial or no response. No major toxicities were observed, and treatment with mesenchymal stem cells seemed to be safe. This therapy may be a promising treatment for corticosteroid-refractory acute GVHD, but further investigation is required.

CHRONIC GVHD

Epidemiology and Risk Factors

Chronic GVHD is the major cause of late non-relapse death following allogeneic HCT [131], and is associated with decreased quality of life and impaired physical and functional status [132]. It remains the most common problem in long-term survivors. However, chronic GVHD has been associated with beneficial GVL/GVT effects [133]. The median time of diagnosis of chronic GVHD is 4.5 months after HLA-identical sibling transplantation and 4

months after unrelated donor transplantation [134]. The incidence of chronic GVHD has been increasing with the wider availability of PBSCs and the increased age of transplant recipients. The development of chronic GVHD ranges from 30% in recipients of fully histocompatible sibling donor transplants to 60% to 70% in recipients of mismatched hematopoietic cells or hematopoietic cells from an unrelated donor. Other factors that likely increase the development of chronic GVHD include prior acute GVHD, older recipient age, female donor (multiparous, in particular) with a male recipient, and use of peripheral blood stem cells [132].

Prevention and Pathophysiology

Although prevention of acute GVHD has improved during the past three decades, no effective prophylaxis regimen currently exists for chronic GVHD. Management of chronic GVHD is guided by a multidisciplinary approach to treatment, including recent adjustment of immunosuppressive medications and aggressive supportive care.

Our understanding of the pathophysiology of chronic GVHD is not as advanced as that of acute GVHD. Chronic GVHD is a complex, multisystem disorder with myriad manifestations that involves many different organs (see below). It is characterized by immune dysregulation, immunodeficiency, impaired organ function and decreased survival [135]. Alloreactive T cells have been implicated in the pathogenesis; however, the precise role of specific T cell subsets, autoantigens, alloantigens, and B cells, as well as interactions of chemokines and cytokines has not been fully elucidated. The clinical manifestations of chronic GVHD are often similar to an autoimmune process, suggesting similar pathophysiology.

Clinical Features

The diagnosis of chronic GVHD is based upon specific signs, degree of organ involvement (mild, moderate, severe), laboratory data, and/or histopathologic confirmation rather than time of onset posttransplant (i.e. > 100 days). Historically, a classification of “limited” (localized skin involvement with or without limited hepatic dysfunction) versus “extensive” (generalized skin involvement or limited disease plus eye, oral, liver, or other target organ involvement) was the most commonly adopted staging system [136]. However, a new consensus criteria for the diagnosis and staging of chronic GVHD has been proposed by the National Institutes of Health Consensus Development Project (Table 3) [105]. Chronic GVHD may present as acute GVHD merging into chronic (progressive type), develop following a period of resolution from acute GVHD (quiescent or interrupted type), or occur de novo. An overlap syndrome includes clinical features in which diagnostic or distinctive features of chronic GVHD and acute GVHD appear together. Specific signs and symptoms, including erythematous skin rash, nausea, vomiting, diarrhea, and liver dysfunction are shared between the two.

Diagnostic Approach

The potential clinical manifestations of chronic GVHD are many and varied, involving multiple organs and sites. At least one distinctive manifestation for chronic GVHD is required to diagnose chronic GVHD, such as oral or vaginal lichenoid findings, ocular sicca,

skin dyspigmentation, scleroderma, or bronchiolitis obliterans. Biopsies or other diagnostic tests (e.g., laboratory tests, radiographic tests, and/or pulmonary tests) are recommended to confirm the diagnosis of chronic GVHD. Definitive diagnosis of chronic GVHD requires excluding other possible diagnoses such as infection, drug effects, malignancies and residual post-inflammatory damage and scarring.

Treatment

Definitive treatment of chronic GVHD, including the implementation and withdrawal of specific therapies, remains elusive. The prior use of prophylaxis agents and/or the history of therapies used in acute GVHD ultimately affect the choice of treatment for chronic GVHD, as do the specific patient characteristics and preferences of the treating physician and center. Generally, treatment for chronic GVHD is less intense and less aggressive than that used for acute GVHD, but may require a prolonged duration of multimodal immunosuppressive therapy. The most widely used first-line therapy for patients with chronic GVHD is a combination of systemic corticosteroids and a calcineurin inhibitor. The recently published National Institutes of Health (NIH) guidelines recommend this treatment if 3 or more organs are involved or any single organ has a severity score of more than 2 [105]. The NIH Working Group defines failure of initial therapy or requirement of additional secondary therapy as follows: i) progression of chronic GVHD despite optimal first-line therapy (including >1 mg/kg/day of prednisolone for two weeks), or ii) no improvement after 4–8 weeks of sustained therapy, or iii) inability to taper corticosteroid dose [105].

Chronic immunosuppressants, especially those containing steroids, are toxic and often result in life-threatening infectious complications. Many second-line therapies for chronic GVHD have been studied, but none has achieved widespread acceptance. Agents studied in chronic GVHD include ECP, MMF, and rituximab. ECP has demonstrated significant response rates in high risk chronic GVHD patients, particularly in the skin, liver, oral mucosa, eye, and lung [137]. However, the response of chronic GVHD to treatment is often unpredictable, and mixed responses in different organs can occur in the same patient. MMF showed initial promise, but in a multicenter placebo-controlled randomized trial, the addition of MMF to the initial systemic treatment regimen for chronic GVHD did not show any benefit [138]. In small phase II trials, rituximab, an anti-CD20 chimeric monoclonal antibody, has demonstrated an overall response rate of 65–70% for its use in refractory chronic GVHD [139, 140]. More recently, it has been shown that patients with fibrotic, chronic GVHD have antibodies activating the platelet-derived growth factor receptor pathway [141]. In this light, a pilot study in 19 patients with refractory chronic GVHD investigated imatinib, which inhibits this pathway. At six months, the overall response rate was 79% with 7 complete remissions and 8 partial remissions [142]. These promising results deserve larger exploration and longer study.

Supportive Care in the Management of GVHD Patients

Supportive care and routine monitoring are critical in the management of chronic GVHD. Early recognition of high-risk features (such as thrombocytopenia, progressive onset chronic GVHD, extensive skin involvement with sclerodermatous features, and multi-organ involvement) and appropriate early intervention are important [105]. The Ancillary Therapy

and Supportive Care Working Group as part of the 2005 NIH-sponsored Consensus Conference in chronic GVHD, recently established guidelines for ancillary and supportive care therapies [137]. Routine interval history, physical examination with assessment of symptoms, review of medications, laboratory monitoring (complete blood cell counts with differential, chemistry panel to assess kidney and liver functions, drug monitoring, IgG levels, lipid profiles, and endocrine evaluations to assess thyroid function, calcium and vitamin D levels) and the consultation of appropriate sub-specialists should be performed. It is important that transplant physicians and primary hematologists recognize the increased incidence of second malignancies in transplant recipients, particularly squamous cell carcinoma in chronic GVHD patients [143]. Furthermore, specific attention to the surveillance of infection prophylaxis (anti-bacterial, -viral, and -fungal), vaccinations, general hygiene, physical therapy, nutritional status, pain control, and monitoring of drug-drug interactions and the associated drug-related side effects, is required. There is little supporting evidence for the routine use of intravenous immunoglobulin as prophylaxis [144], but patients should receive routine antibiotic prophylaxis (penicillin or its equivalent) due to the increased risk of streptococcal sepsis [145]. The sites of any indwelling catheter should be assessed regularly. Early recognition of signs or symptoms of septic shock requires prompt evaluation with blood cultures and initiation of broad spectrum antibiotics.

Future Directions

The population of patients with GVHD has steadily grown as the number of allogeneic transplantations being performed annually has increased. Newer treatment approaches and wider availability of supportive care and ancillary therapies have led to improved survival. Despite these recent advances, however, GVHD remains a leading cause of morbidity and mortality in survivors of allogeneic transplantation. Novel approaches to GVHD are thus urgently needed. In this light, it is important to recognize that the management of patients with GVHD is complex and requires a multidisciplinary approach. A major barrier to current therapies is life-threatening infectious complications due to broad non-specific immune suppression. Future strategies are likely to include modulation of cell types that play key roles in the GVHD process, including regulatory T cells, dendritic cells, NKT cells and B cells. As such, targeted therapies are preferable in order to preserve the beneficial anti-tumor effect while eliminating the debilitating consequences of GVHD. Identification of biomarkers for GVHD with diagnostic and prognostic significance may further clarify groups of patients at highest risk and eventually improve the management of GVHD.

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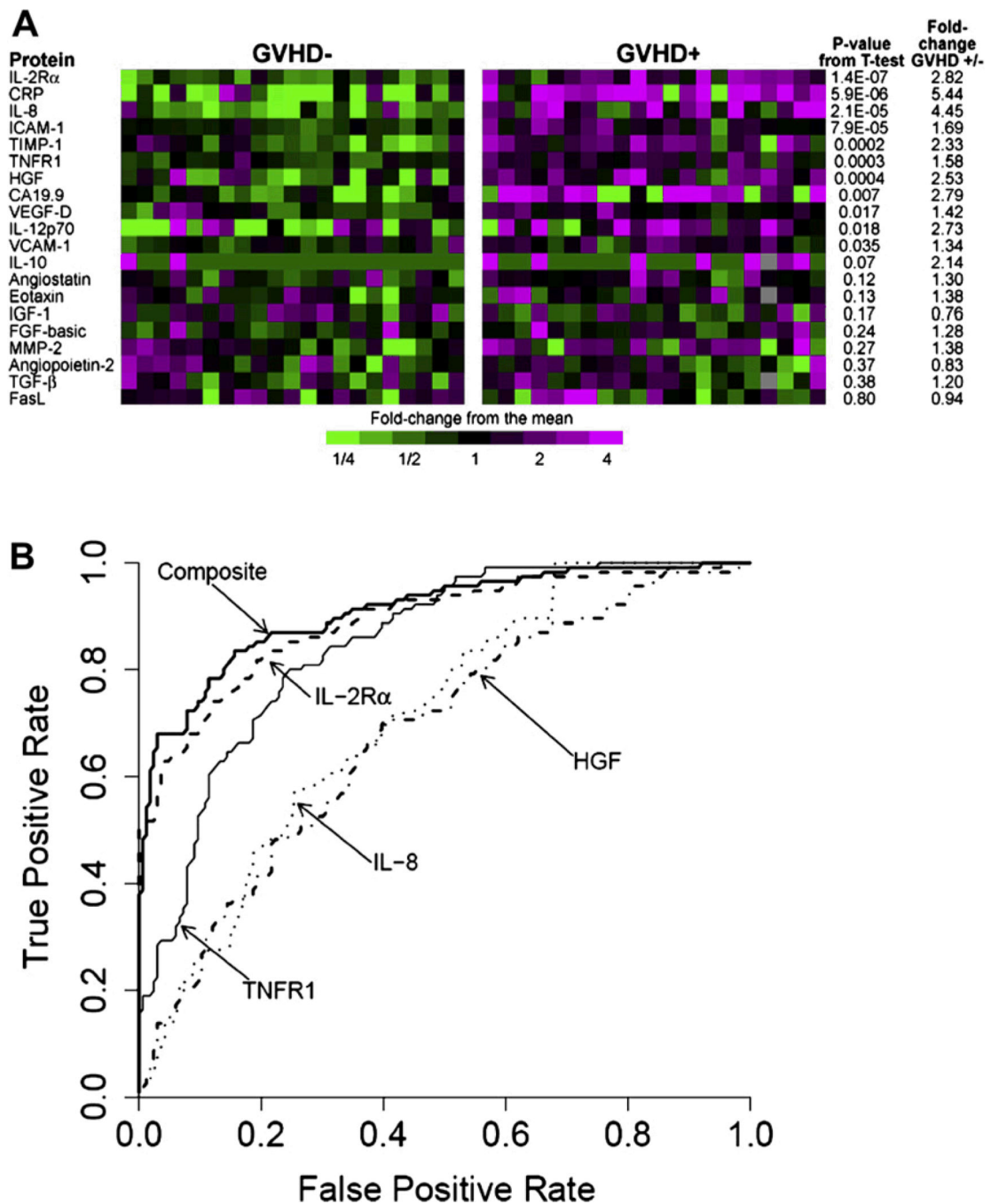


Figure 1. From discovery to validation of plasma biomarkers of acute graft versus host disease (This research was originally published in *Blood*. [115])

Panel A shows the heatmap of proteins levels measured sequential ELISA in the discovery set samples. Samples from 21 GVHD- patients (left) and 21 GVHD+ patients (right) are represented. Eleven proteins gave a P values for differences between GVHD+ and GVHD- patient plasma < 0.05. Panel B shows the receiver operating characteristic (ROC) curves of

four individual discriminator proteins and the composite panel in the training set. Individual ROC curves for IL-2Ra, TNFR1, HGF, and IL-8 and the composite panel.

Table 1
Acute GVHD Grading Criteria

The standard GVHD grading criteria was developed by Glucksberg in 1974 [146], and then revised at the 1994 Consensus Conference in Keystone [147]. The grading system did not initially take into consideration the staging of the GI tract for pediatric patients. However, most pediatric centers have adopted the modified Glucksberg grading scale and adjusted the staging of the GI tract to be based on volume per kilogram of body weight.

Stage	Skin	Liver (bilirubin)	GI tract (stool output/day)
0	No GVHD rash	< 2 mg/dl	Adult: < 500 ml/day Child: < 10 ml/kg/day
1	Maculopapular rash < 25% BSA	2–3 mg/dl	Adult: 500–999 ml/day Child: 10–19.9 ml/kg/day or persistent nausea, vomiting, or anorexia, with a positive upper GI biopsy
2	Maculopapular rash 25–50% BSA	3.1–6 mg/dl	Adult: 1000–1500 ml/day Child: 20–30 ml/kg/day
3	Maculopapular rash > 50% BSA	6.1–15 mg/dl	Adult: >1500 ml/day Child: > 30 ml/kg/day
4	Generalized erythroderma with bullous formation	> 15 mg/dl	Severe abdominal pain with or without ileus

BSA = body surface area, GI = gastrointestinal, GVHD = graft versus host disease.

Overall Clinical Grade
Grade

-
- 0 No stage 1–4 of any organ
 - 1 Stage 1–2 skin rash and no liver or GI involvement
 - 2 Stage 3 skin rash, or Stage 1 liver involvement, or Stage 1 GI involvement
 - 3 Stage 0–3 skin rash, with Stage 2–3 liver involvement, and/or Stage 2–3 GI involvement
 - 4 Stage 4 skin rash, liver, and/or GI involvement
-

Table 2
Selected studies of novel agents as secondary or first line therapy in acute GVHD

Novel Therapy	Type of trial	Type of treatment	Sample size	Overall Response at 4 weeks	Survival at 3–6 months	Study	Reference
Denileukin difitox	Phase I	steroid refractory	30	71%	33%	Ho et al.	[148]
Denileukin difitox	Phase II	steroid refractory	22	41%	ND	Shaughnessy et al.	[149]
Denileukin difitox	Phase II randomized	Initial therapy	60	60%	49%	Alousi et al.	[122]
Etanercept	Phase II	Initial therapy	61	69%	69%	Levine et al.	[150]
Etanercept	Phase II randomized	Initial therapy	60	48%	56%	Alousi et al.	[122]
Pentostatin	Phase I	steroid refractory	23	78%	ND	Bolanos-Meade et al.	[151]
Pentostatin	Phase II randomized	Initial therapy	60	62%	52%	Alousi et al.	[122]
MMF	Phase I/II	steroid refractory	48	72%	78%	Basara et al.	[152]
MMF	Phase I/II	steroid refractory	10	60%	70%	Krejci et al.	[153]
MMF	Phase I/II	steroid refractory	6	67%	64%	Takami et al.	[154]
MMF	Phase II randomized	Initial therapy	60	78%	64%	Alousi et al.	[122]
Sirolimus	Phase I/II	steroid refractory	21	57%	28%	Benito et al.	[155]
BDP	Phase II randomized	Initial therapy	129	71%	87%	Hockenbery et al.	[119]
ECP	Phase II	steroid refractory	59	68%	ND	Greimix et al.	[125]
ECP	Phase II	steroid refractory	23	52%	48%	Perfetti et al.	[126]
ECP	Phase II	steroid refractory	41 (children)	73%	100%	Calore et al.	[127]
ECP	Phase II	steroid refractory	15 (children)	0% for grade IV GVHD	ND	Berger et al.	[128]
MSC	Phase II	steroid refractory	55	71%	ND	Le Blanc et al.	[130]
MSC	Phase II	steroid refractory	13	15%	31%	von Bontin et al.	[156]
Alefacept	Phase I	steroid refractory	16	75%	ND	Shapira et al.	[157]

GVHD indicates graft versus host disease, MMF, mycophenolate mofetil, BDP, beclomethasone dipropionate, ECP, extracorporeal photopheresis, MSC, mesenchymal stromal cell

Table 3

Signs and Symptoms of Chronic GVHD (reproduced with permission) [105]

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features*	Common (Seen with Both Acute and Chronic GVHD)
Skin	Poikiloderma Lichen planus-like features Morphea-like features Lichen sclerosus-like features	Depigmentation	Sweat impairment Ichthyosis Keratosis pilaris Hypopigmentation Hyperpigmentation	Erythema Maculopapular rash Pruritis
Nails		Dystrophy Longitudinal ridging, splitting, or brittle features Onycholysis Pterygium unguis Nail loss (usually symmetric; affects most nails) [†]		
Scalp and body hair		New onset of scarring or nonscarring scalp alopecia (after recovery from chemoradiotherapy) Scaling, papulosquamous lesions	Thinning scalp hair, typically patchy, coarse, or dull (not explained by endocrine or other causes) Premature gray hair	
Mouth	Lichen-type features Hyperkeratotic plaques Restriction of mouth opening from sclerosis	Xerostomia Mucocele Mucosal atrophy Pseudomembranes [†] Ulcers [†]		Gingivitis Mucositis Erythema Pain
Eyes		New onset dry, gritty, or painful eyes [‡] Cicatricial conjunctivitis Keratoconjunctivitis sicca [‡] Confluent areas of punctate keratopathy	Photophobia Periorbital hyperpigmentation Blepharitis (erythema of the eyelids with edema)	
Genitalia	Lichen planus-like features Vaginal scarring or stenosis	Erosions [†] Fissures [†] Ulcers [†]		
GI tract	Esophageal web Strictures of stenosis in the upper to mid third of the esophagus [†]		Exocrine pancreatic insufficiency	Anorexia Nausea Vomiting Diarrhea Weight loss Failure to thrive (infants and children) Total bilirubin, alkaline phosphatase > 2×upper limit of normal [†] ALT or AST > 2×upper limit of normal [†]
Lung	Bronchiolitis obliterans	Bronchiolitis obliterans diagnosed with PFTs and radiology [†]		BOOP

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features*	Common (Seen with Both Acute and Chronic GVHD)
	diagnosed with lung biopsy			
Muscles, fascia, joints	Fasciitis Joint stiffness or contractures secondary to sclerosis	Myositis of polymyositis [‡]	Edema Muscle cramps Arthralgia or arthritis	
Hematopoietic and immune			Thrombocytopenia Eosinophilia Lymphopenia Hypo- or hypergammaglobulinemia Autoantibodies (AIGA and ITP)	
Other			Pericardial or pleural effusions Ascites Peripheral neuropathy Nephrotic syndrome Myasthenia gravis Cardiac conduction abnormality or cardiomyopathy	

GVHD indicated graft-versus-host disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BOOP, bronchiolitis obliterans-organizing pneumonia; PFTs, pulmonary function tests; AIGA, autoimmune hemolytic anemia; ITP, idiopathic thrombocytopenic purpura.

* Can be acknowledged as part of the chronic GVHD symptomatology if the diagnosis is confirmed.

[†] In all cases, infection, drug effects, malignancy, or other causes must be excluded.

[‡] Diagnosis of chronic GVHD required biopsy or radiology confirmation (or Schirmer test for eyes).