Reprint of: B Cells in Chronic Graft-versus-Host Disease ☆

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A B S T R A C T

Chronic graft-versus-host disease (cGVHD) continues to be a common complication of allogeneic hemato-poietic stem cell transplantation. Unlike acute graft-versus-host disease, which is mediated almost entirely by donor T cells, the immune pathology of cGVHD is more complex and donor B cells have also been found to play an important role. Recent studies from several laboratories have enhanced our understanding of how donor B cells contribute to this clinical syndrome and this has led to new therapeutic opportunities. Here, Dr Sarantopoulos reviews some of the important mechanisms responsible for persistent B cell activation and loss of B cell tolerance in patients with cGVHD. Dr Blazar describes recent studies in preclinical models that have identified novel B cell–directed agents that may be effective for prevention or treatment of cGVHD. Some B cell–directed therapies have already been tested in patients with cGVHD and Dr Cutler reviews the results of these studies documenting the potential efficacy of this approach. Supported by mechanistic studies in patients and preclinical models, new B cell–directed therapies for cGVHD will now be evaluated in clinical trials.

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INTRODUCTION

Chronic graft-versus-host disease (cGVHD) after alloge-neic hematopoietic stem cell transplantation (HSCT) con-tinues to be a common, debilitating, and deadly complication of therapy. Despite improved tools for diagnosis and clinical assessment of disease activity, cGVHD pathophysiology remains ill defined and this has hampered the development of effective new therapies [1,2]. In this regard, analysis of patient blood and tissue samples and new murine models of cGVHD have expanded our knowledge of disease patho-genesis and the complexity of mechanisms that lead to tissue damage [3]. Although donor T cells clearly play a critical role in the initiation and maintenance of allo-immunity, many laboratory and clinical studies have shown that donor B cells also play an important role in the pathophysiology of cGVHD [4-6]. Importantly, therapeutic strategies targeting B cells can provide clinical benefit in many patients with active cGVHD [7].

This review will focus on recent advances in our under-standing of the role of B cells in cGVHD. A series of new studies in HSCT patients and murine models have begun to elucidate the role of B cells in the pathogenesis and persis-tence of cGVHD and this has led to the evaluation of new therapeutic approaches specifically targeting aspects of B cell reconstitution and function after HSCT. As these new thera-peutic approaches are evaluated and integrated with other established therapies, we anticipate that new therapeutic agents will lead to significant improvement in the long-term outcome of patients with cGVHD.

B CELL ACTIVATION PATHWAYS IN CHRONIC GVHD

In healthy individuals, B cell development is a dynamic, daily process with a high propensity for the formation of self-reactive B cells. Despite central B cell tolerance mechanisms, a remarkably large pool of polyreactive and potentially auto-reactive B cells arise at a constant rate from bone marrow (BM) precursor cells [8]. Receptor editing, deletion, and anergy induction in the BM [9-11] do not eliminate all potentially auto-reactive B cell clones, and it has been esti-mated that 55% to 75% of transitional B cells emerging from BM in healthy adults are self-reactive [8,12]. The mainte-nance of normal B cell immunity, therefore, requires deletion of auto-reactive clones coupled with positive selection after encounter with microbes (or other foreign antigens) [13]. In conjunction with B cell receptor (BCR) signaling, B cell–activating factor (BAFF) plays an important role in
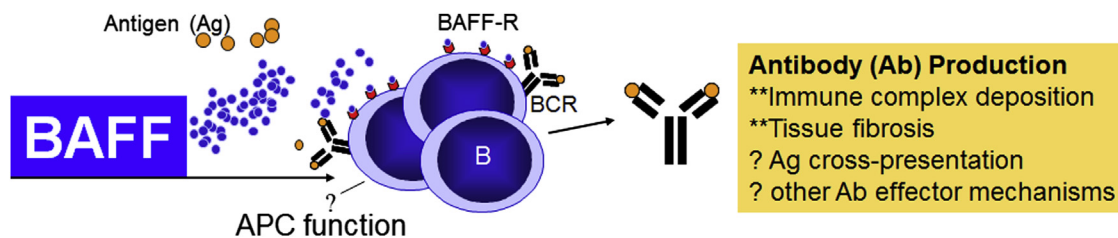
Figure 1. BAFF and antigen-driven B cell activation. In cGVHD, B cells are promoted by antigen and excessive BAFF levels to survive and produce antibodies. Constitutive antibody production by certain B cell subsets in cGVHD may directly mediate pathology. B cells may also serve as antigen presenting cells (APC).

determining B cell fate/survival. In acquired autoimmune diseases, abnormally high levels of BAFF subvert the development of B cell tolerance by attenuating BCR-triggered apoptosis of polyreactive B cells. In self-reactive BCR transgenic murine models, limiting amounts of BAFF are required to promote B cell turnover and avoidance of auto-reactivity [14,15]. Early after HSCT, the peripheral B cell compartment likely comprises recent BM emigrants consisting of short-lived transitional cells. Although these cells are capable of primary immune reactions and generate short-lived plasma cells, they do not take part in the germinal center (GC) reaction. This likely explains why B cell populations after HSCT have a relatively low diversity of antigen binding sites (ie, BCRs) with a high frequency of low-affinity, potentially allo- or auto-reactive antibodies. As BAFF levels are high after HSCT, B cells that are not deleted through negative selection are likely positively selected during B cell recovery. Although specific antigen targets remain largely unknown, high-throughput BCR sequencing of B cell subsets suggests that the IgG CDR3s comprise poly- and auto-reactive characteristics [16]. These data, along with frequent production of auto-antibodies [17-19], suggest a critical breakdown in peripheral B cell tolerance in patients with cGVHD. Taken together, these findings suggest a model for aberrant persistence of allo- and auto-reactive B cells after transplantation, shown in Figure 1. In this model, we hypothesize that as B cells develop after HSCT, there is a failure of normal B cell tolerance checkpoints, at least in part due to high levels of BAFF. As a result, there is persistence of donor B cells reactive to a variety of recipient antigens and secretion of pathologic allo- and auto-antibodies.

In the presence of a large, diverse, mature B cell pool, normal B cells consume and sequester BAFF, and auto-reactive B cell clones that require high levels of BAFF are unable to survive [20]. After allogeneic HSCT, persistently elevated BAFF levels are associated with B lymphopenia and cGVHD development [5,21]. In contrast, supranormal B cell numbers are found in patients without cGVHD [21,22]. Because B cells in patients without cGVHD have high levels of BAFF receptors, these cells are able to sequester BAFF and prevent high levels of BAFF from promoting auto-reactive B cell clones [23]. This hypothesis is supported by the finding that patients unable to robustly recover their B cell compartment may not respond to rituximab, and BM production of B cells early after HSCT may be critical for prevention of cGVHD [21,22,24,25]. In addition to mature B cells that compete for BAFF, IL-10—producing regulatory B cells may play an important role in the prevention of cGVHD [26]. IL-10—producing cells are present in higher proportions in patients without cGVHD [27]. Our data indicate a role for altered B cell homeostasis in cGVHD pathogenesis and reveal a limitation of anti-CD20, rituximab, therapy after HSCT [28]. How the B cell compartment is skewed toward robust

mature B cell recovery and perhaps IL-10 production requires further study.

To determine if B cells are activated in cGVHD, we examined unstimulated B cell lysates for signaling pathways known to be downstream of BAFF. B cells in cGVHD showed evidence of increased metabolic activity and survival, with enhanced signaling via the AKT and ERK pathways [29]. BIM knockout mice have an autoimmune phenotype that is independent of BAFF, and BIM counteracts BAFF signaling [30]. Accordingly, excess BAFF reduces BIM levels in transgenic BCR auto-reactive murine B cells [31]. Although increased AKT and ERK signaling may be BCR-dependent, proapoptotic BIM is also increased after BCR engagement unless counteracted by BAFF. Consistent with BAFF-associated AKT and ERK signaling, we found BIM isoforms were decreased in cGVHD B cells [32]. Thus, similar to what is found in autoimmune mice, high BAFF levels may rescue intermediate affinity, self-reactive B cells during recovery after HSCT by attenuating apoptosis of recirculating B cells after GC reactions [14,15]. Chronic exposure to BAFF results in hypermetabolic, enlarged B cells, capable of constitutive auto-antibody production [33]. It is therefore tempting to directly link excessive BAFF levels to B cell activation in cGVHD pathophysiology, but this link remains to be determined.

In addition to BAFF, B cell activation and survival depend on the affinity and specificity for antigen and on coreceptor molecule binding for conveyance of positive or negative BCR signals [34]. We have begun to investigate this further in cGVHD to identify potential therapeutic molecular targets. In healthy mice, BAFF sets the threshold for BCR-mediated selection among naïve, mature B cells [35]. In the absence of BAFF, tonic or chronic stimulation with antigen results in constant signaling through the BCR, leading to anergy or death [34]. In the setting of constant exposure to foreign (or allo) antigens and excessive BAFF, B cell hyper-responsiveness to antigen may contribute to cGVHD pathogenesis. Using anti-IgM as a surrogate for soluble antigen, we found that cGVHD B cells not only responded more robustly after exposure, but that this response was associated with increased protein levels of the proximal BCR machinery molecules spleen tyrosine kinase (SYK) and B cell linker protein (BLNK) [36]. Proliferation after antigen engagement has been mechanistically linked to both BAFF-R and BLNK [37,38]. Signaling after BCR engagement by antigen entails phosphorylation and activation of the tyrosine kinase SYK, which subsequently phosphorylates and activates the tyrosine-rich adapter protein BLNK that is then recruited to the BCR signalosome. BLNK is central to further downstream signaling, as it also acts as a docking protein for Bruton's tyrosine kinase (BTK), PLCγ2, and the other crucial BCR complex signaling molecules. We found that this heightened activation via BCR was abrogated by the SYK inhibitor fostamatinib [36], suggesting potential clinical utility of this
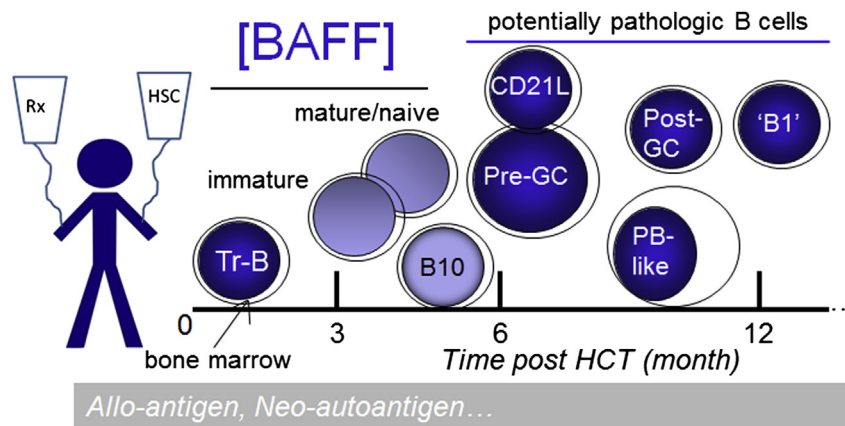
Figure 2. Development and persistence of potentially pathological cGVHD B cells. Altered B cell homeostasis with high BAFF to naïve B cell ratios after HCT leads to persistence of aberrantly activated B cells. Decreased bone marrow output and production of immature (and potentially of B10) B cells leads to excessive levels of BAFF, sufficient to support potentially pathological B cell subsets.

agent. Further experiments in murine models of cGVHD in collaboration with Dr. Blazar's lab have also shown the importance of BCR signaling and the ability of SYK inhibition to reverse tissue damage associated with cGVHD. Together, our data suggest that BCR signaling after antigen engagement during B cell reconstitution after HSCT is likely critical for persistence of auto-reactive B cells, and selective inhibition of BCR signaling may provide a new therapeutic target in patients with cGVHD.

Although genetic disparity between donor and recipient must exist for cGVHD to develop, transferable T cell auto-reactivity occurs after allo-reactivity in murine models [39-41]. In patients, antibodies to both allo-antigens and to nonpolymorphic ("auto") antigens are also found. For example, a coordinated T-B response to disparate epitopes on the minor histocompatibility antigen DBY has been described [42,43]. T cells remain crucial effector cells in this disease [44], and although B-T coordinated responses have been delineated [42,43], whether B cells drive T cell responses remains unclear. Antibodies to polymorphic and nonpolymorphic recipient antigens have been associated with cGVHD [4,17,18]. In addition to other B cell functions [40,45,46], secreted antibodies likely play a role in cGVHD tissue destruction, since Ig deposits have been observed in lesional skin tissue [6,47]. Likewise, antibody production was recently shown in a murine model to be required for cGVHD development [6]. Studies in other diseases demonstrate the role of antibodies in cross-presentation to T effector cells [48,49]. Thus, allo- and auto-antibodies may facilitate the cross-presentation of antigens to T effector cells, thereby amplifying T cell responses to minor histocompatibility antigen or auto-antigens [49,50]. Unrestrained T follicular helper (TFH) cells have been associated with loss of B cell tolerance, potentially in abnormal GC reactions [51,52]. In mice, TFH cells are vital for increased GC responses and for cGVHD genesis [53]. In patients, GC composition is altered, but whether TFH cells promote aberrant B cells is an area of active investigation. Cell surface CD27 increases after B cells encounter antigen. We found that circulating CD27^{+} B cells from cGVHD patients constitutively produce IgG without the requirement of further BCR or second signal stimulation [5]. These cells also have a distinct gene expression profile compared with healthy CD27^{+} B cell counterparts [32]. In patients, both pre-GC and post-GC peripheral blood B cell subsets occur in increased proportions, and these increases were associated with augmented levels of plasma BAFF [5]. Thus, unlike the antimicrobial memory response of healthy individuals, cGVHD CD27^{+} B cells potentially mediate anti-normal recipient tissue responses, although further study is required to affirm and quantify the pathologic potential of these cells [54,55].

Together, our data have allowed us to develop a working model for potentially disease-mediating B cell development after HSCT (Figure 2). As aberrantly activated cells represent potential therapeutic targets, further studies of B cell signaling in patients with cGVHD are clearly needed. Pursuit of hypothesis-driven studies of human B cell function will advance our understanding of cGVHD pathophysiology and will lead to development of clinical trials aimed at prevention and treatment.

PRECLINICAL MODELS EVALUATING NOVEL B CELL–DIRECTED THERAPIES FOR CHRONIC GVHD

Preclinical development of new therapeutic modalities for cGVHD has been hindered by models with pathologic findings that may not simulate the development of human cGVHD. We developed a multi-organ system model of cGVHD induced by allogeneic HSCT after a conditioning regimen of cyclophosphamide and total body radiation. In this model, cGVHD manifestations are observed in a wide spectrum of target organs (lung, liver, spleen, thymus, colon, tongue) [6,56]. Fibrosis was demonstrated in the lung and liver and was associated with CD4^{+} T cells, B220^{+} B cell infiltration, and IgG2c class-switched antibody deposition [6]. Lung fibrosis resulted in pulmonary dysfunction and airway obliteration, which leads to bronchiolitis obliterans syndrome (BOS), pathognomonic for cGVHD of the lung. Antibody secretion from BM-derived B cells was essential for cGVHD, as evidenced by the lack of disease when using BM incapable of producing B cells or secreted polyclonal IgG. GCs, critical for efficient class switching and antibody secretion, were significantly increased in size and number in cGVHD mice [6]. cGVHD maintenance was dependent upon GC formation, as infusion of a lymphotoxin-receptor-immunoglobulin fusion protein into mice with established cGVHD reduced GCs and suppressed BOS measured by pulmonary function tests.

GCs are formed by cooperation between a subset of T and B cells known as TFH cells and GC B cells, respectively. Recently, we reported that increased frequency of TFH cells

correlated with increased GC B cells, cGVHD, and BOS. In patients, the anti-CD20 mAb, rituximab, can prevent steroid-requiring chronic GVHD as assessed in a phase 2 trial [57] and results in amelioration of cGVHD in patients, especially those with skin and mucosal involvement [7]. However, not all patients respond and not all responses are permanent. We have found that administering a highly depleting anti-CD20 mAb to mice with established cGVHD resulted in peripheral B cell depletion, but B cells remained in the lung, BOS was not reversed [53], and cGVHD manifestations remained [58]. Instead, BOS could be treated by eliminating production of IL-21 by donor T cells or IL-21 receptor (IL-21R) signaling of donor B cells. Blocking mAbs for TFH-GC B cell interactions, including IL-21/IL-21R, inducible T cell costimulator/inducible T cell costimulator ligand, and CD40L/CD40, hindered GC formation and cGVHD, indicating a role for TFH and GC B cells and suggesting a new line of therapy to reverse cGVHD.

To study transcriptional pathways and develop targeted therapeutics for cGVHD, we studied the effects of emerging therapeutic agents targeting GCs. BCL6 is a master regulatory transcription factor that facilitates GC development in a cooperative manner with emerging chromatin-associated factors, namely the EZH2 lysine methyltransferase and the BRD4 epigenetic reader protein [59,60]. To determine whether strategies that disrupt GC integrity could be used to treat cGVHD we targeted BCL6 using a direct-acting ligand, 79-6; [61,62], EZH2 using 3 structurally distinct inhibitors (JQ-E, UNC1999 [63] and DZNep [64]); and a first-in-class BRD4 inhibitor, JQ1 [60,65]. In T cells, *Bcl6* is selectively expressed in TFH and GC Tregs. We observed a 10-fold increase in percent of splenic BCL6$^+$ T cells in cGVHD mice. The small molecule BCL6 inhibitor 79-6 was synthesized and used to treat mice with active cGVHD. Treatment reversed BOS and resulted in a decrease in GC B cells and lung collagen. EZH2 catalyzes the methylation of lysine 27 on histone 3 (H3K4me3) silencing genes to a transcriptionally repressive state. EZH2 is markedly upregulated during the GC reaction and prevents GC B cell terminal differentiation, allowing affinity maturation to occur [62,66,67]. Selective EZH2 deletion in either BM-derived B cells or splenic T cells completely prevented cGVHD with a decrease in GC frequency. Whereas naïve T cells express low EZH2 levels, EZH2 is rapidly upregulated upon allo-stimulation. We compared 3 drugs that reduce H3K4me3, 2 pyridinone inhibitors (JQ-E and UNC1999) and DZNep, which destabilizes EZH2 complexes. At established doses, DZNep and UNC1999 were ineffective or toxic, respectively. In contrast, JQ-E fully reversed cGVHD lung dysfunction and fibrosis around the bronchioles was significantly decreased.

JQ1 is a first-in-class epigenetic reader that recognizes histone modifications and has been shown to reduce B cell lymphomas via inhibiting super-enhancer—associated transcripts and to treat cardiac failure—induced fibrosis. JQ1 administration to cGVHD mice significantly inhibited BOS and collagen deposition. Taken together, these data demonstrate for the first time the critical role of BCL6 and targeting of histones that affect the transcriptional repressive states via EZH2 and a BET bromodomain epigenetic reader. These data also provide a strong foundation for clinical trials of inhibitors that directly or epigenetic modifiers and readers that indirectly target BCL6.

Given our data on TFH produced IL-21 in cGVHD, we tested a Rho-associated kinase 2 inhibitor, KD025, that blocks IL-21 production [68,69] and has been shown safe in normal healthy volunteers. KD025 inhibits STAT3 phosphorylation, which supports Th17 generation and IL-21 production, each of which we have linked to cGVHD generation in our model. Concurrently, KD025 increases STAT5 phosphorylation and regulatory T cell suppressor function in a dose-responsive fashion. KD025 treatment shifts Th17/regulatory T cell balance. KD025-treated cGVHD mice had a dose-dependent decrease in the development of pathogenic pulmonary function, which correlated with a marked reduction of antibody deposition in the lungs compared with non-cGVHD controls, along with a decrease in collagen deposition in the lungs and GCs. We demonstrated that mice that underwent transplantation with inducible STAT3-deficient T cells or BM cells had pulmonary function comparable to the healthy controls, suggesting that STAT3 is a potential therapeutic target in both T and B cells and is necessary for the development of cGVHD.

Many of the cellular activation and effector functions of Th17 and TFH cells can be attributed to BTK and IL-2 inducible kinase (ITK) [70,71] and antibody production by GC B cells depends upon BTK [71]. Ibrutinib is a first-in-class irreversible inhibitor of BTK and ITK that blocks downstream immune receptor activation [72-74]. Because ibrutinib can block the activation of B cells via BTK inhibition as well as specific T-helper subsets that drive the development of cGVHD via ITK inhibition, we hypothesized that it may be ideally suited to the treatment of cGVHD. We show that ibrutinib treatment reversed TFH-GC—dependent cGVHD and BOS, associated with blockade of GC formation and reduction of antibody deposition and fibrosis. Additionally, ibrutinib ameliorated the progression of cGVHD in a minor antigen, T cell—dependent murine model of sclerodermatous cGVHD, reducing skin lesions, hair loss, and lymphohistiocytic infiltration [75]. These data strongly support the clinical investigation of ibrutinib as a novel therapeutic strategy for the treatment of cGVHD.

SYK is activated by BCR engagement. After antigen-BCR engagement, SYK is phosphorylated at Y348, allowing for B cell survival and proliferation. Increased proximal BCR signaling was recently described in cGVHD patient B cells [36]. We now demonstrate that SYK was hyperactive in B cells during cGVHD and that SYK expression was necessary in B but not T cells for murine cGVHD progression. We found that total B cell frequency in mice receiving SYK knock-out (KO) BM was decreased 8-fold, whereas T cell frequency was unaffected. These data are consistent with the dependency of activated B cells on SYK for proliferation and survival and a requirement for activated donor-derived BM B cells in cGVHD pathogenesis [6,53].

Fostamatinib is a potent small molecule inhibitor of SYK. Studies in rheumatoid arthritis have demonstrated efficacy with fostamatinib in randomized phase II clinical trials [76,77]. Mice receiving fostamatinib for cGVHD treatment had restoration of pulmonary function, similar to the healthy controls that had undergone transplantation, along with a reduced number of GC reactions in the spleen associated with a decrease in frequency of splenic GC B cells. To determine if human cGVHD B cells are more susceptible to SYK blockade, B cells purified from human peripheral blood were treated in vitro with R406, the active form of R788. B cells from patients with cGVHD had increased apoptosis compared with patients without cGVHD after R406, consistent with work by Allen et al. [36] that revealed that R406 preferentially kills cGVHD B cells via apoptosis. Together, these data now demonstrate that constitutively activated B cells can be selectively targeted in cGVHD.

Table 1
Monoclonal Antibodies, Drugs, and Their Targets Used to Treat Established cGVHD in Mice

Agent	Target
Anti-IL21	IL21/IL21R
Anti-ICOS	ICOS/ICOSL
Anti-CD40L	CD40/CD40L
79-6	BCL6 peptidomimetic
JQ-E	EZH2
JQ-1	BET bromodomain
KD025	ROCK2
Ibrutinib	BTK, ITK
Fostamatinib	SYK

ICOS indicates inducible T cell costimulator; ROCK2, Rho-associated kinase 2.

In summary, we have developed an array of antibody and drug targeting approaches based upon preventing effective TFH-GC interactions (Table 1). As many of these reagents have been tested in patients, these data lay the foundation for repurposing such reagents to treat steroid-refractory cGVHD in the clinic.

B CELL–DIRECTED THERAPY FOR TREATMENT AND PREVENTION OF CHRONIC GVHD

Standard immune-suppressive therapies for established cGVHD have primarily targeted the T cell compartment. With this approach, responses tend to be incomplete, and the toxicity of long-term administration of corticosteroids is prohibitive. Second-line anti–T cell therapy results in even poorer outcomes. The first anecdotal evidence that targeting B cells could be effective in the treatment of established GVHD was provided by Ratanatharathorn et al. [78], and Miklos et al. [4,79] were the first to associate the formation of specific anti-HY antibodies in a sex-mismatched model with the occurrence of cGVHD (and protection from relapse). Since then, a number of B cell–dependent processes have been implicated in the pathogenesis of cGVHD, and numerous clinical studies have provided evidence that targeting B cells can be effective in the management of and prevention of cGVHD.

Treatment of Corticosteroid-Refractory cGVHD

Several groups have reported the consistent effectiveness of B cell depletion as therapy for established advanced cGVHD [80]. In a review of the published literature on the effectiveness of rituximab for the treatment of corticosteroid-refractory cGVHD, response rates ranging from 43% to 83% were reported with a pooled overall response rate of 66% (95% confidence interval [CI], 57% to 74%) [81]. Common to all reported studies, rituximab administration in subjects with established cGVHD is relatively safe with a low incidence of adverse events. However, long-term replacement immunoglobulin therapy is often required. There appears to be a predilection for higher response rates in individuals with sclerodermatous or lichenoid cutaneous disease [82] and so a randomized trial comparing rituximab with imatinib, another agent with activity against cutaneous sclerosis, has recently been completed by the Chronic GVHD Consortium.

Initial Therapy of cGVHD

Sahaf et al. performed a pilot study of rituximab in combination with corticosteroids as initial therapy of cGVHD [83]. Thirty-five subjects with new-onset cGVHD who required corticosteroid treatment received 4 weekly infusions of rituximab (375 mg/m^2/week) in combination with prednisone (1 mg/kg/day). A second course was allowed for incomplete responses. Response, defined as a complete or partial response with a prednisone dose of <.25 mg/kg/day, was noted in 40% of subjects at 6 and 12 months, suggesting promising but incomplete activity. Although there were 6 deaths from cGVHD within 12 months of rituximab therapy, only 2 subjects suffered a relapse of their malignancy [83]. In this trial, cGVHD responses were predicted by a naïve (CD19$^+$CD38$^-$IgD$^+$CD27$^-$) B cell phenotype ($P = .03$). Fifteen cGVHD subjects included had undergone female to male (F→M) HSCT, and 6 (38%) were H-Y IgM positive whereas 9 (56%) were IgG positive at cGVHD diagnosis. Among the 15 female to male HSCT recipients with cGVHD treated with rituximab, 11 responded, and both H-Y IgM and IgG became undetectable for at least 6 months after rituximab. In contrast, the 4 F→M subjects who did not respond remained H-Y IgG positive. Moreover, 2 subjects who redeveloped H-Y IgG later had recurrent cGVHD. This suggests that either the allo-reactive B cell or the antibodies elucidated by plasma cell derivatives from these cells may actually be pathogenic.

Prevention of cGVHD

Given the promising role for B cell depletion as therapy for established cGVHD, we explored the utility of rituximab therapy for primary prevention of cGVHD [57]. In a phase II trial, rituximab (375 mg/m^2) was administered at 100 days and 6, 9, and 12 months after HSCT to 65 subjects without evidence of cGVHD at study onset. The cumulative incidence of cGVHD and systemic corticosteroid-requiring cGVHD at 2 years from HCT were 48% and 31%, respectively, lower than rates in a concurrent control cohort (60%, $P = .10$ and 48.5%, $P = .015$, respectively). In related donors, the incidence of cGVHD and corticosteroid-requiring cGVHD at 2 years was 35% and 23%, whereas the corresponding incidence in unrelated donors was 59% and 38%. Rituximab was safe, with no severe infusion-related events and a 2-year cumulative incidence of grade 3 to 5 infections of 15%. There was no difference in relapse incidence (34% versus 28%, $P = .79$), but treatment-related mortality at 4 years from HSCT was significantly lower in rituximab-treated subjects when compared with controls (5.1% versus 19.0%, $P = .02$), and overall survival was superior at 4 years (71% versus 56%, $P = .05$). In a multivariable regression model, only rituximab use (hazard ratio, .56; 95% CI, .31 to 1.00; $P = .048$) and high/very high disease risk index (hazard ratio, 1.90; 95% CI, 1.02 to 3.53; $P = .04$) were significantly associated with overall survival. B cell leukopenia was profound and persisted through 18 months from HSCT, when evidence of B cell reconstitution was evident in some subjects. The BAFF to B cell ratio was significantly higher in cGVHD subjects in comparison with cGVHD-free subjects at 2 years ($P = .039$). This trial included 13 F→M recipients, none of whom developed anti-DBY antibodies between 6 months and 1 year from HSCT, whereas 4 of 10 F→M control recipients developed anti-DBY antibodies in the same time period ($P = .02$).

In a similar experience, Arai et al. also tested the role of rituximab as primary prevention of cGVHD after HSCT using a total lymphoid irradiation (TLI)/antithymocyte globulin (ATG) preparative regimen [84]. In a small trial of 35 subjects, rituximab (375 mg/m^2) was infused weekly on days 56, 63, 70, and 77 after HSCT. The cumulative incidence of cGVHD was 20%, which is slightly lower than the published rates of cGVHD after TLI/ATG based transplantation [85]. In 10 F→M

recipients enrolled in this trial, there was complete prevention of allo-reactive H-Y antibody development, and none of these subjects developed cGVHD. In comparison, among 25 F→M HCT patients who used the same TLI/ATG conditioning regimen but without prophylactic rituximab during this time period, 14 of the 25 (56%) developed H-Y antibodies, with 13 of the 25 (52%) developing cGVHD ($P = .01$).

Most recently, Glass et al. reported the results of a randomized trial in which 42 subjects with non-Hodgkin lymphoma were randomized to receive rituximab (375 mg/m^2) on 8 separate days between weeks 3 and 28 after transplantation [86]. Thirty-six subjects actually received 1 or more doses, but in an intention-to-treat analysis, there was no difference in the incidence of cGVHD (33% in rituximab-treated patients versus 41% in control patients, $P = .28$). However, in this trial, ATG was used, reducing the global rates of cGVHD, and the relapse rate was high early after transplantation (10 subjects in each group relapsed at a median of 88 days and 90 days in the rituximab and control groups, respectfully), making a competing risk cumulative risk analysis less statistically powerful to detect differences.

Earlier attempts to prevent GVHD in single-arm studies (generally to prevent acute GVHD, reviewed in Kharfan-Dabaja and Cutler [7]) as well as a recent experience with B cell depletion to prevent Epstein-Barr virus reactivation [87], did not demonstrate a marked effect on the incidence of cGVHD; however, in the majority of these experiences, B cell depletion was administered early in the peri-transplantation period, before active B cell reconstitution.

CONCLUSIONS AND OTHER CONSIDERATIONS

Anti–B cell therapy has been firmly established as being partially effective in the treatment of established cGVHD and recent studies suggest that anti–B cell therapy may also play a role in the primary prevention of cGVHD. However, control and prevention with anti-CD20 monoclonal antibody, rituximab, is incomplete, and novel strategies are required to improve outcomes and prevent the recurrent cGVHD that can occur with B cell reconstitution. Novel second generation anti-CD20 monoclonal antibodies may provide more effective B cell depletion and elimination of pathogenic allo- and auto-reactive antibodies in vivo. However, as demonstrated in preclinical models, small molecule inhibitors that target the BCR signaling complex may be more effective than rituximab. Clinical studies evaluating this approach will require testing in prospective clinical trials and have recently begun. Additionally, anti–B cell therapy in combination with T cell–directed therapies may ultimately be the most effective strategy.

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