B-cell targeting in chronic graft-versus-host disease

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Over the last decade, our understanding of the pathophysiology of chronic graft-versus-host disease (cGVHD) has improved considerably. In this spotlight, we discuss emerging insights into the pathophysiology of cGVHD with a focus on B cells. First, we summarize supporting evidence derived from mouse and human studies. Next, novel cGVHD therapy approaches that target B cells will be covered to provide treating physicians with an overview of the rationale behind the emerging armamentarium against cGVHD. (*Blood*. 2018;131(13):1399-1405)

Introduction

Chronic graft-versus-host disease (cGVHD) is a major complication in patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT), leading to reduced patient-reported quality of life[1] and nonrelapse mortality.[2] Risk factors for cGVHD development include prior acute GVHD, donor peripheral blood stem-cell grafts, HLA disparity, female donors for male recipients, and recipient age.[3] Clinical cGVHD can involve classical acute GVHD epithelial target tissues (intestinal tract, liver, skin, lung) and any other organ system, including oral, esophageal, musculoskeletal, joint, fascial, ocular, hair and nails, lymphohematopoietic system, and genital tissues.[4] The pleiotrophic symptoms resulting from such broad organ involvement made past diagnosis and scoring difficult. The 2005 and revised 2014 National Institutes of Health (NIH) criteria have brought greater consistency to terminology and methods for cGVHD diagnosis and staging.[4,5]

To identify and validate novel targets in cGVHD, numerous mouse models are used. However, individual cGVHD mouse models cannot reproduce all features of cGVHD seen in patients (as reviewed by Zeiser and Blazar[6,7]), who present with a heterogeneous disease spectrum. Most models have 1 or 2 dominant cGVHD manifestations involving limited numbers of organs. These different manifestations of cGVHD depend on several factors, including the cytokines that are released. Some of these cytokines or their receptors are attractive targets to treat cGVHD. For instance, an anti–interleukin 2 (IL-2) receptor common γ chain neutralizing monoclonal antibody (mAb) reduced cGVHD,[8] a result that may be based on a broad inhibitory effect on multiple cytokine receptors. Also, targeting of individual cytokines such as IL-17 was active against cGVHD.[9] Additionally, the type and degree of donor and recipient genetic disparity in models suggest that the antigens recognized by B and T cells as well as the number of donor T cells transferred can dictate cGVHD phenotypes. Thus, mechanistic studies of multiple models when feasible are ideal.[10]

The role of B cells in cGVHD based on findings in mice

Under normal conditions, B cells contribute to adaptive immunity by producing antibodies, secreting cytokines, and presenting antigen. B-cell activation begins when an antigen is recognized via the B-cell receptor (BCR). Activated B cells participate in a 2-step differentiation process that yields both short-lived plasmablasts for immediate protection against a pathogen and long-lived plasma cells and memory B cells for persistent protection.[11] Together with BCR signaling, B-cell activating factor (BAFF) determines B-cell fate/survival. Comparable to the normal B-cell activation process, the first step in the pathogenesis of cGVHD is the recognition of antigen via the BCR (Figure 1A step 1). In contrast to the normal situation, B cells exhibit BCR hyperresponsiveness in cGVHD as shown in mouse models.[12-14] After activation, pathogenic B cells expand (Figure 1A step 2) and are strongly affected by soluble factors in the microenvironment such as IL-4, IL-17,[9] IL-21,[12,15] and BAFF[16] (Figure 1A step 3). This process is connected to the formation of GCs in cooperation with donor Tfhs. GC B cells undergo somatic hypermutation that can favor cGVHD by increasing the frequency of B cells capable of producing antibody to antigens that trigger BCR.

IL-4 produced by CD4 T cells promotes B-cell immunoglobulin isotype switching,[17-19] allowing daughter cells from the same activated B cell to produce secreted pathogenic IgG in cGVHD mice.[12,17] Tfhs produce IL-21, which can promote auto- and alloreactive B-cell activation and survival along with increased local BAFF levels in cGVHD.[16] Although the role of GCs in cGVHD initiation is likely to be important in many cGVHD mouse models, GCs were found not to be required for disease development in a recent report,[20] possibly reflecting the wide clinical spectrum of cGVHD in patients. In a consecutive step, activated B cells can promote tissue injury via antibody and cytokine production and release, leading to clinical manifestations of cGVHD (Figure 1A step 4). IgG-induced macrophage activation may contribute to cGVHD via secretion of proinflammatory cytokines such as IL-6 and IL-22,[21]
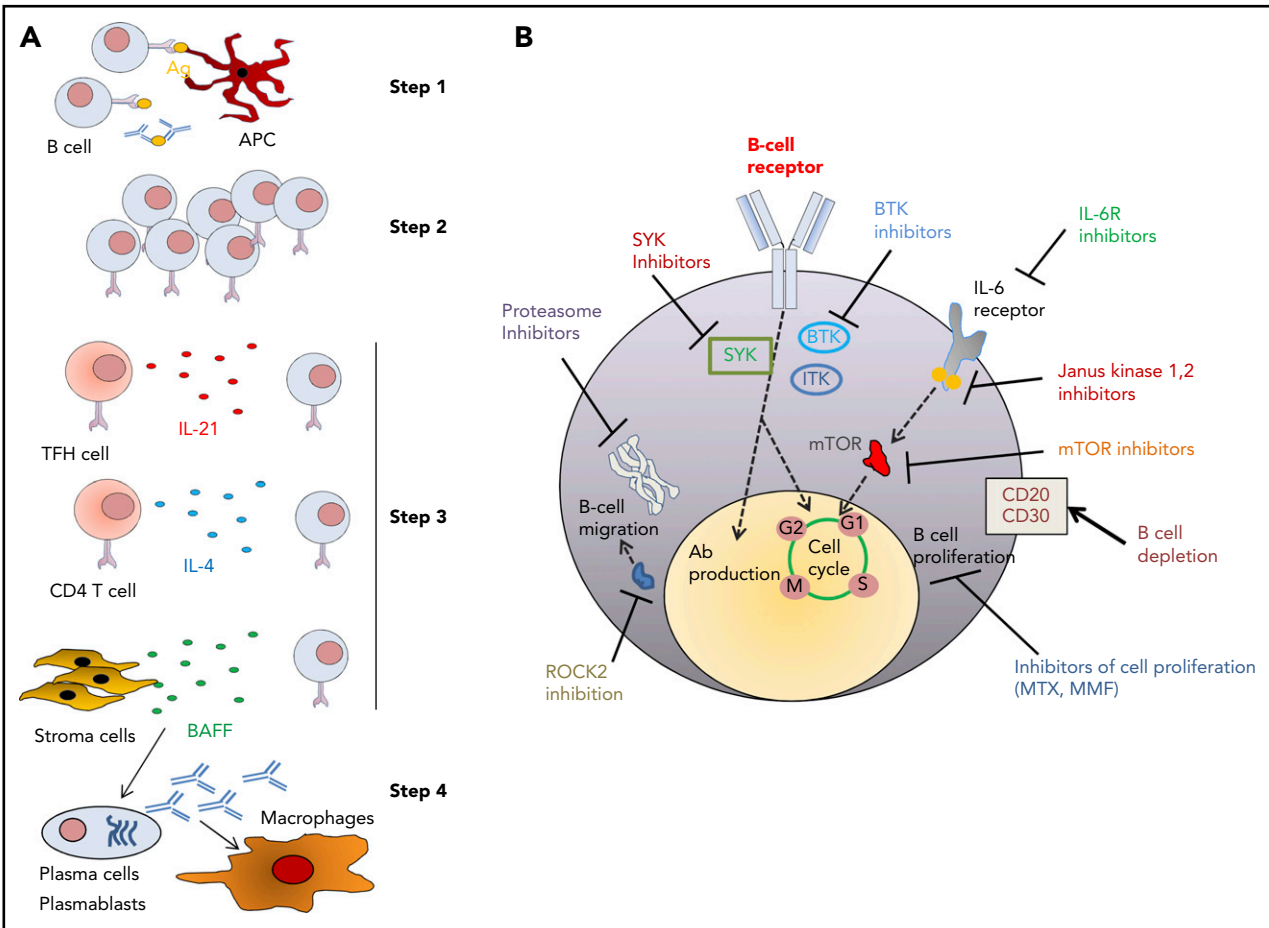
Figure 1. The role of B cells in cGVHD. (A) Different steps of cGVHD development. Step 1: antigen (Ag)-presenting cells (APCs) present auto- and alloantigens and prime B cells. Direct activation of B cells via Ag or Ag/Ab complexes. APCs prime B cells against major histocompatibility complexes/peptides or neoantigens (eg, Y chromosome–encoded genes). This is enhanced in certain B-cell subgroups by hyperreactive BCR signaling. In addition to B-cell activation by APCs, there is likely also direct BCR activation via Ag or Ab/Ag complexes. Step 2: expansion of auto- and alloreactive B cells. Step 3: activated T follicular helper cells (Tfhs) produce IL-21 and cell-surface costimulatory molecules that lead to germinal center (GC) formation, which is not counterbalanced by sufficient T follicular regulatory cells (Tfrs). CD4 T helper cells produce IL-4, which promotes Ab class switch in autoreactive B cells. Stroma cells produce BAFF, which promotes B-cell activation. Step 4: plasma cells and plasma blasts produce high amounts of immunoglobulin. Deposition of immunoglobulin G (IgG) can lead to macrophage activation and organ damage. IgG-induced macrophage activation may contribute to cGVHD via secretion of proinflammatory cytokines by macrophages such as IL-6, which promotes B-cell survival and maintains inflammation. (B) Strategies to target B cells in cGVHD. The sketch shows a B cell and the mode of action of multiple immunosuppressive strategies that directly act on B cells or plasma cells in the context of cGVHD. The summary of translation of each approach is provided in Table 1. BTK, Bruton tyrosine kinase; ITK, IL-2–inducible kinase; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; MTX, methotrexate; ROCK2, ρ-GTPase kinase-2; SYK, splenic tyrosine kinase.

which maintain inflammation. Tissue stiffness in cGVHD can be enhanced by copious immunoglobulin production and deposition together with fibroblast-derived extracellular matrix molecules including collagen and proteoglycans (Figure 1A step 4).

The role of B cells in cGVHD: evidence from studies on human tissues

Pathogenic B-cell activation is found in various autoimmune diseases including systemic lupus erythematosus, multiple sclerosis, rheumatoid arthritis, type 1 diabetes, and others as well as in cGVHD.[17,19] During cGVHD, donor B cells and T cells mount a coordinated response to both allogeneic and autologous antigens, which leads to their expansion (Figure 1A steps 1 and 2). Allogeneic antigens include minor histocompatibility antigens[22,23] that are typically expressed or processed intracellularly and presented as peptides by major histocompatibility complex

molecules. These include Y chromosome proteins/peptides in male recipients of female donor grafts, as well as cell membrane antigens, the former correlating with cGVHD by multivariable logistic regression analysis.[24] Autoantigens are antigens on donor hematopoietic cells, which can be found for example on megakaryocytes or platelets. In agreement with the concept of recognition of autoantigens, patients can develop autoimmune thrombocytopenia after allo-HCT, which is mediated by antibodies produced by donor B cells and directed against donor platelets.

BAFF promotes B-cell survival and activation (Figure 1A step 3) and is significantly increased in plasma of patients with cGVHD.[25,26] BAFF and BCR-associated signaling work in concert to promote activation and survival of B cells from patients with cGVHD.[27] In those with cGVHD, B cells exhibit increased BCR responsiveness[27] via increased proximal BCR intracellular signaling molecules SYK and B-cell linker (BLNK).[28] In that context,

it is important to understand which cell-intrinsic mechanisms enhance BCR responses. A novel observation here is that BCR responses to surrogate antigen were markedly increased when NOTCH2 was also activated.[29] Intrinsic differences in important transcription factors like IRF4 contributed to NOTCH2 expression and responsiveness. How extrinsic factors like BAFF and intrinsic molecular pathways like NOTCH promote BCR-activated B cells is currently not clear but is an area of active investigation.

Tfhs can support antihost antibody production.[30] This process typically takes place in GCs, areas of lymph nodes where B cells are activated in mice,[30] but where this occurs in cGVHD in patients remains unknown. As in patients with autoimmune disease, this process also may occur in extrafollicular locations. Antigen targets of B-cell responses in cGVHD remain largely unknown, but ultimately, both auto- and alloimmune B-cell responses can occur. Lack of sufficient T regulatory cells (Tregs) in patients with cGVHD can contribute to impaired peripheral tolerance.[31] Tregs are capable of selectively killing B cells,[32] and their deficiency would predispose to a failure to control pathogenic B cells. Although human memory Tregs expand after allo-HCT, they cannot compensate for the lack of naïve Tregs, because of short telomeres and increased apoptosis.[33] cGVHD tissue stiffness and organ dysfunction are likely supported by cooperation between B cells and macrophages, leading to fibroblast activation; however, so far there is no direct evidence for this interaction (Figure 1A step 4).

Impaired central and peripheral tolerance mechanisms in cGVHD

Under homeostatic conditions, multiple mechanisms prevent pathogenic B-cell function via central (thymic) and peripheral tolerance. In patients undergoing allo-HCT, uncontrolled expansion and immunoglobulin production by B cells possibly occurs because of thymic dysfunction. Impaired thymic function is caused by aging, conditioning regimen toxicity, calcineurin inhibitors, alloreactive T cells, and immunoglobulin deposition.[19,34] Alloreactive T cells contribute to the process by depleting thymic dendritic cells, medullary thymic epithelial cells (TECs), and cortical TECs.[34,35] A recent report also suggests pathologic antibodies target TECs in a cGVHD model.[15,18] GVHD affects both positive selection by cortical TECs and negative selection by thymic B cells and cortical TECs,[34,36] which allows potentially pathogenic CD4^{+} T cells to escape from tolerization or deletion before peripheral export[37,38] and impedes the development of Tregs that contribute to peripheral tolerance.

Mouse studies revealed that peripheral immune tolerance to recipient tissues after transplantation is mediated by Tregs, Tfrs representing Tregs that migrate to the GCs,[12] regulatory B cells,[39] type 1 regulatory T cells,[40] and invariant natural killer T cells.[41-43] Tregs and Tfrs negatively regulate B-cell responses and cGVHD,[43] and B regulatory cells that release IL-10 have been shown to ameliorate sclerodermatous cGVHD severity.[44] In agreement with these mouse studies, analysis in patients with cGVHD suggests that B cells with a regulatory phenotype are both decreased and inactive.[39,45] Increased T-cell help decreases self-regulation by B cells by promoting aberrant B-cell generation. Additionally, the absence of robust recovery of the peripheral B-cell compartment results in excess BAFF and

promotion of autoreactive B cells that can cooperate to overwhelm peripheral tolerance mechanisms in those with cGVHD.[46] Additionally, thymic T-cell generation, negative selection of antihost reactive T cells, thymic Treg production, and peripheral Treg survival are severely reduced in patients with cGVHD.[31,47,48]

Novel and early-phase therapeutic strategies that target B cells in cGVHD

B-cell depletion with anti-CD20 antibodies was performed in preclinical models and patients.[12,49,50] Anti-CD20 mAbs administered in the prophylactic setting reduced murine cGVHD, whereas established cGVHD was nonresponsive.[12,49] In the clinical setting, the anti-CD20 mAb rituximab conferred some efficacy in patients with steroid-refractory cGVHD (SR-cGVHD),[51] with attenuation of cGVHD in those patients who robustly recovered B cells.[46,52] A prospective phase 2 trial showed that naïve B cells (PD-L1[hi]) were significantly reduced at cGVHD diagnosis but increased after rituximab treatment.[50] To target plasma cells, different drugs that have been successfully used in the treatment of multiple myeloma such as pomalidomide[53] were tested in cGVHD (Table 1; Figure 1B). IL-6 was shown to contribute to cGVHD. Because IL-6 is known to promote plasma blast and plasma-cell survival,[54] further study of IL-6 and B cells is warranted. The anti–IL-6 receptor mAb tocilizumab is being investigated in a clinical trial as therapy for cGVHD.[55] In other diseases, IL-6 also has a known role in promotion of collagen deposition and extracellular matrix production by fibroblasts.[56]

Several small-molecule inhibitors are now in the pipeline, building upon the observation that patients with cGVHD have hyperreactive BCR signaling via the BCR proximal tyrosine kinase SYK. SYK was found to be upregulated in cGVHD B cells in mice[12,13] and patients.[28] SYK inhibition reduced established murine cGVHD, was associated with reduced GC responses, and activated CD80/86[+] dendritic cell responses[10] and induced apoptosis in B cells of patients with cGVHD.[10,13,28] On the basis of these promising findings, the SYK inhibitor entospletinib, recently granted US Food and Drug Administration (FDA) orphan drug status, is being studied as first-line treatment with steroids.[57] Further downstream of the BCR is BTK. In B cells of patients with cGVHD, phosphorylated BTK was present in the absence of in vitro stimulation by anti-IgM.[14] In agreement with a role of BTK, cGVHD severity was reduced in murine recipients receiving donor B cells lacking BTK or ibrutinib that targets BTK.[14] Ibrutinib additionally inhibits ITK,[14] and in a cGVHD model where T cells lacked ITK, cGVHD was reduced.[14] On the basis of these findings, it is likely, but not formally proven, that both BTK and ITK inhibition are critical to the efficacy of ibrutinib in cGVHD. In patients with cGVHD, ibrutinib reduced murine sclerodermatous and multiorgan system cGVHD as well as T- and B-cell activation.[14,58] Guided by these preclinical data, an open-label phase 2 study evaluated the safety and efficacy of ibrutinib in patients with active cGVHD with SR-cGVHD.[59] At a median follow-up of 13.9 months, best overall response was 67% (sustained ≥20 weeks in 71% of responders).[59] On the basis of these clinical data and upon the foundations of the applied NIH consensus criteria from 2005, ibrutinib was FDA approved for SR-cGVHD.

With better understanding of the role of B cells in cGVHD pathogenesis, multiple additional strategies have been developed that

Table 1. Targeting B cells in cGVHD

Target name	Normal function	Name of drugs tested	Species analyzed	Evidence for role in cGVHD	Reference
BTK and ITK	Downstream of BCR activation	Ibrutinib	Mouse	Yes	14,58
			Human	Yes	59
CD20	B-cell surface antigen	Rituximab	Mouse	Yes (effective only in prevention)	49
			Human	Yes	46,50-52
CD30	B cells express CD30	Brentuximab	Human	Clinical trials ongoing	74
IL-6 receptor	IL-6 induces proliferation of pre–B cells	Tocilizumab	Human	Clinical trials ongoing	55
JAK 1/2	JAK1/2 mediate downstream effects of cytokine and chemokine receptors in B cells[63]	Ruxolitinib	Mouse	Yes	64
			Retrospective clinical data	Yes	64
			Prospective phase 3 trial ongoing	Clinical trials ongoing	65
Plasma cells	Production of immunoglobulin that causes organ damage in cGVHD	Pomalidomide	Human	Clinical trials ongoing	53
Proteasome	Activation of the proteasome is important in plasma cells	Bortezomib	Mouse	Yes	75
			Human	Clinical trials ongoing	76
Proteasome	Activation of the immunoproteasome is important in plasma cells	Carfilzomib	Human	Clinical trials ongoing	77
ROCK2	T-cell activation with pSTAT3 and pSTAT5 effects; B-cell migration	KD025	Mouse	Yes	61
				Decreases Tfhs	
			Human	Clinical trials ongoing	62
SYK	Downstream of BCR activation; Cell migration; Endocytosis	Entospletinib; Fostamatinib	Mouse	Yes	10,13
				Clinical trials ongoing	57

Presented in alphabetical order.

ZEISER et al

deplete B cells, reduce their activation via manipulation of BCR-downstream events, or inhibit their migration toward inflammatory sites. Other agents also potentially target cytokine-mediated B-cell differentiation or survival. In normal mice and healthy volunteers, in vitro Tfh generation depends upon the ROCK2.[60] In both murine sclerodermatous and multiorgan system cGVHD models, ROCK2 inhibition with KD025 ameliorated ongoing cGVHD, was associated with reduced Tfhs resulting from inhibition of pSTAT3 and IL-21 production, and increased Tfrs as a result of augmentation of pSTAT5 signaling.[61] A phase 2a KD025 trial to treat SR-cGVHD[62] is ongoing. BCR stimulation also activates JAK2/STAT3 signaling.[63] In mice, JAK1/2 blockade with ruxolitinib inhibited multiple murine cGVHD features.[64] Clinical responses were reported in a survey of patients with SR-cGVHD treated with ruxolitinib.[64] On the basis of these promising results, a phase 3 multicenter ruxolitinib trial for treating SR-cGVHD[65] is in progress. How the B-cell compartment is affected by these agents is unclear.

Pirfenidone inhibits TGF-β receptor signaling; downregulates NLRP3 inflammasomes, growth factors, and procollagen I and II; and is FDA approved for treating idiopathic pulmonary fibrosis. Pirfenidone treatment of established murine cGVHD restored pulmonary function and reversed lung fibrosis and was associated with reduced pulmonary macrophage infiltration and TGF-β production.[66] How B cells are affected by agents that block fibrotic pathways requires further investigation.

Autoreactive B-cell regulation is mediated via Tregs. Tregs have the capacity to control recipient reactive B cells, with their expansion and survival dependent upon IL-2 production by T effector cells.[67] Thus, low-dose IL-2 infusion has been tested as cGVHD treatment. A phase 1/2 study showed that exogenous IL-2 increased Tregs and improved cGVHD.[68,69] On the basis of the defects in Tregs reported for patients with cGVHD, [31,48] a clinical study analyzed the feasibility and efficacy of human expanded Tregs administered to patients with cGVHD.[70] The study reported that 2 of 5 treated patients achieved a complete remission.

Summary and outlook

Recent advances in our understanding of the role of B cells in cGVHD pathogenesis have paved the way for novel strategies that target activation, expansion, survival, and Ab production of B cells. Studies are urgently needed, because the first-line gold standard for cGVHD therapy remains steroids, which have multiple severe adverse effects. Both mouse and human studies of B-cell pathways have been a major driver in testing the aforementioned novel therapies. These drugs were in some instances already clinically applied in other diseases. In spite of their potential clinical benefit, an important clinical consideration is that cGVHD is connected to overall reduction in relapse.[71] Thus, overly intensive cGVHD prevention may lead to reduced graft-versus-leukemia activity. Clinical judgment, the application of the NIH criteria for cGVHD diagnosis and scoring,[4,72] novel cGVHD biomarkers,[73] and measurement tools will be essential to make clinical meaningful progress in cGVHD treatment via B-cell targeting.

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