CHRONIC GRAFT-VERSUS-HOST DISEASE

Chronic graft-versus-host disease: biological insights from preclinical and clinical studies

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With the increasing use of mismatched, unrelated, and granulocyte colony-stimulating factor–mobilized peripheral blood stem cell donor grafts and successful treatment of older recipients, chronic graft-versus-host disease (cGVHD) has emerged as the major cause of nonrelapse mortality and morbidity. cGVHD is characterized by lichenoid changes and fibrosis that affects a multitude of tissues, compromising organ function. Beyond steroids, effective treatment options are limited. Thus, new strategies to both prevent and treat disease are urgently required. Over the last 5 years, our understanding of cGVHD pathogenesis and basic biology, born out of a combination of mouse models and correlative clinical studies, has radically improved. We now understand that cGVHD is initiated by naive T cells, differentiating predominantly within highly inflammatory T-helper 17/T-cytotoxic 17 and T-follicular helper paradigms with consequent thymic damage and impaired donor antigen presentation in the periphery. This leads to aberrant T- and B-cell activation and differentiation, which cooperate to generate antibody-secreting cells that cause the deposition of antibodies to polymorphic recipient antigens (ie, alloantibody) or nonpolymorphic antigens common to both recipient and donor (ie, autoantibody). It is now clear that alloantibody can, in concert with colony-stimulating factor 1 (CSF-1)-dependent donor macrophages, induce a transforming growth factor β–high environment locally within target tissue that results in scleroderma and bronchiolitis obliterans, diagnostic features of cGVHD. These findings have yielded a raft of potential new therapeutics, centered on naive T-cell depletion, interleukin-17/21 inhibition, kinase inhibition, regulatory T-cell restoration, and CSF-1 inhibition. This new understanding of cGVHD finally gives hope that effective therapies are imminent for this devastating transplant complication. (Blood. 2017; 129(1):13-21)

Introduction

Chronic graft-versus-host disease (cGVHD) remains the major cause of morbidity and nonrelapse mortality after allogeneic hematopoietic stem cell transplantation (SCT).1-3 Progress in improving cGVHD prevention and therapy has been hindered by complexities in cGVHD diagnosis and staging,4-5 lack of uniform treatment response criteria,6 paucity of controlled trials,7 and access to new therapies with an established proof-of-concept or strong pathophysiological basis in preclinical models. Such progress has been supported by analysis of human materials acquired from cGVHD patients.

This review draws from animal model and clinical studies to provide an overview; we combined interpretation of our current understanding of the cellular and molecular mediators of cGVHD. In turn, we highlight promising new therapeutic approaches. Additionally, we will provide our perspective on the gaps in cGVHD basic biology that deserve more attention as the prevalence of clinical cGVHD grows. Finally, we will review translation of current and possible future cGVHD therapies that have evolved from cGVHD basic biological insights.

Because no individual review can cover all aspects of cGVHD pathogenesis and preclinical studies leading to clinical applications, the reader is referred to several excellent reviews on this subject.5-13 Mouse models have served as a mainstay for recent advances in cGVHD therapies, and hence, will be a focus of this review. As virtually all patients receive some form of conditioning, nonconditioned murine cGVHD models will not be discussed in this review; instead, we refer the reader to Chu et al.9

CGVHD manifestations and initiating factors in the clinic

cGVHD typically manifests with multiorgan pathology and historically has been defined temporally as GVHD that occurred later than 100 days post-SCT. The commonly seen diagnostic features, as outlined by the National Institutes of Health (NIH) consensus criteria,14 include skin pathology varying from lichen planus–like lesions to full sclerosis, bronchiolitis obliterans (BO), and oral lichen planus–like lesions (ie, skin, lung, and mouth involvement). Esophageal webs and strictures and muscle or joint fasciitis are also diagnostic. Importantly, these diagnostic features can be seen before day 100 and may occur simultaneously with features commonly seen in acute GVHD (aGVHD) (eg, macular-papular rashes, weight loss, diarrhea, and hepatitis). Thus, cGVHD occurs as a continuum in time with clinical features that are distinct from, but not mutually exclusive with, those seen in aGVHD.
Over the last decade granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cell (G-PBSC) grafts have been rapidly adopted as an increasingly used stem cell source for SCT. From its inception, it was clear that G-CSF exerts immunomodulatory effects on the graft,15-17 resulting in altered transplant outcomes in patients receiving G-PBSC grafts as compared with unmanipulated bone marrow (BM) grafts, with the primary advantage of G-PBSC grafts being accelerated engraftment. A randomized trial of BM vs G-PBSC revealed similar overall survival with secondary end points showing that G-PBSC grafts provided decreased graft failure but increased cGVHD incidence.18,19 Consistent with G-CSF immune-regulatory effects on PBSCs, aGVHD incidence was similar despite the higher T-cell dose that accompanied G-PBSC grafts. Risk factors for cGVHD development include preceding aGVHD, use of PBSCs,18 use of mismatched or unrelated donors (as opposed to matched siblings), transplant of female donors to male recipients, absence of antithymocyte globulin in conditioning, and older recipients.20 Given the expanding allogeneic SCT and G-PBSC graft use as well as the treatment of older recipients who historically were not candidates for allogeneic SCT, it is not surprising that the prevalence of cGVHD has reached new heights.

Overview of mouse models and cGVHD pathogenesis

With clinical cGVHD heterogeneity and frequent preceding aGVHD manifestations, it is somewhat surprising that GVHD models in mice have been described in the literature with such a clear demarcation as representing aGVHD or cGVHD. Similar to patients, it is now clear that transplanted mice receiving pre-SCT conditioning regimens, typically radiation-containing, can progress through a continuum of aGVHD to cGVHD which can evolve over time.21 In fact, autoreactive T cells can coexist with or emerge from alloreactive T cells.22-24 Indeed, many aGVHD model systems have been adapted to infuse lower donor T-cell numbers25-27 or use G-CSF treatment of donors,28 permitting mice to escape uniform aGVHD lethality and donor T cells to chronically receive T-cell receptor (TCR) signals from host or donor alloantigen/peptide-expressing cells. Features of cGVHD can be seen in most “aGVHD” models if T-cell doses are lowered and histopathology is later post-SCT (eg, at 4-8 weeks), the latter time favoring both escape from aGVHD lethality and a period of chronic TCR signaling.28

A noteworthy distinction between the pathology of aGVHD and cGVHD is the typical tissue inflammatory T-cell infiltrate and destructive features of aGVHD and the relatively acellular, fibroproliferative findings in cGVHD. In particular, scleroderma,15,16,22,23 BO,25 and fibrosis in liver, gastrointestinal tract, salivary glands, and tongue can be seen in cGVHD mouse models.26,27,29 Intriguingly, many, but not all, cGVHD appear to have either scleroderma (reviewed in Reddy et al30) or multiorgan system involvement without scleroderma as their predominant manifestations, further highlighting the fact that no single model can replicate the wide spectrum of clinical manifestations which themselves are not all seen in an individual patient. There are no unique strain combinations that only cause cGVHD and are incapable of experiencing aGVHD if conditions are modified to favor aGVHD. Because aGVHD can attack the thymus, BM, and secondary lymphoid organs (SLOs), preceding aGVHD, even at a subclinical level in mice and patients, may have profound immunological manifestations resulting in T-cell or especially B-cell depletion31-33 or loss of thymic function34,35 that results in failed negative selection and loss of regulatory T-cell (Treg) production (see “Immune regulators of cGVHD”).23,36-38 Conversely, strategies that prevent or treat cGVHD may be efficacious if they inhibit aGVHD, whereas in settings in which aGVHD is no longer present, successful cGVHD therapy approaches must focus on reversing fibrosis, if debilitating, and any ongoing immune mechanisms that continue to propagate the cGVHD injury response.

Further complicating the analysis of cGVHD pathogenesis using preclinical models are the specifics of cGVHD generation. As in patients, variables that can contribute to differences in cGVHD pathogenesis and its manifestations between laboratories include radiation dose/source/dose rate, use of chemotherapy, subset and numbers of infused donor T cells, and hematopoietic stem cell (HSC) source and manipulations, if any. Other key variables may include mouse vendors39 and distinct microbiome colonization as well as antibiotic usage in each mouse colony that has been shown to affect immune responses,40,41 including aGVHD in mice42,43 and humans.44 Recipient age may be a factor as older mice have augmented allostimulatory function.45 Although in most cGVHD models, donor and host strains are sex-matched, if this is not the case, anti-HY responses could occur with female-into-male transplants potentially resulting in aGVHD in rodents46 and cGVHD in patients.47,48 In our opinion, there is no inherent predilection for cGVHD per se or scleroderma generation in minor histocompatibility antigen (miH)-only disparate models, though such strain combinations are frequently used for analysis of cGVHD pathogenesis. Rather, we favor the explanation that the intensity of the GVHD response and responding T-cell type (CD4 vs CD8 subset and differentiation stage, cytokine profile, chemokine/integrin expression levels) are the major determining factors for aGVHD vs cGVHD independent of the type (major histocompatibility complex [MHC] and/or miH) of antigenic disparities between donor and host. This hypothesis is supported by the fact that miH only as well as models in which MHC antigen disparities are present each have been reported to induce aGVHD and cGVHD, dependent upon transplant conditions. Therefore, our collective recommendation is for the field to focus on discussing the immunological and pathophysiological mechanisms that result in cGVHD, not the system used. As such, we have chosen not to summarize particular strain combinations and SCT conditions that have been reported to cause cGVHD that is typically a part of such reviews.

Relationship between aGVHD and cGVHD pathogenesis

The initiation of and resultant target organ injury observed in both aGVHD and cGVHD is a consequence of the coordinated interplay between multiple cellular and molecular immune mediators that is dependent on the presence and function of donor graft T cells.49 Following SCT, tissue injury and inflammation characterized by proinflammatory cytokine release (eg, tumor necrosis factor [TNF], interleukin-6 [IL-6], and IL-1) is initiated by the conditioning regimen that would be common for both aGVHD and cGVHD, especially in the clinic, as both diseases can emanate in patients who receive the transplantation procedure. These cytokines, together with luminal damage-associated molecular patterns and pathogen-associated molecular patterns released from damaged gut tissue and the microbial luminal contents, result in the activation of antigen-presenting cells (APCs). Activated APCs then prime naive donor T cells and preferentially drive T-helper 1 (Th1)/T-cytotoxic 1 (Tc1) and Th17/Tc17 differentiation and
expand T-effector cells, which can mediate target tissue GVHD, including the thymus and SLO, as well as the skin, liver, gastrointestinal tract, and lung, likely predisposing to cGVHD later after SCT.

Whereas aGVHD is generally defined as a Th1/Th17 paradigm, which results in extensive tissue destruction characterized by apoptosis, cGVHD and aGVHD in fact may share initiating mechanisms. For example, Th17/Tc17 cells have been shown, in some but not all systems, to cause either aGVHD\(^{50-52}\) or sclerodematous cGVHD.\(^{28,53}\) Although donor natural killer (NK) cells, Tregs, regulatory B cells (Bregs), and macrophages play important roles in dampening both aGVHD and cGVHD (see “Immune regulators of cGVHD”), the role of B cells in controlling aGVHD pathogenesis in murine models is more controversial.\(^{54-56}\) In cGVHD, there is a preponderance of evidence for an interplay between donor T cells and donor B cells for disease pathogenesis (see “Immune regulators of cGVHD”). In this section, we define the contribution of each of these mediators to cGVHD pathology as instructed by preclinical studies with confirmation in the clinical setting where applicable.

**Thymic and peripheral T-cell selection defects resulting in cGVHD**

The donor T-cell compartment is composed of antigen-inexperienced naïve and antigen-experienced T-effector and memory subsets. In both preclinical and clinical studies, naïve T-cell-depleted grafts have a significantly reduced cGVHD incidence, while allowing transferred memory T cells to contribute to immune reconstitution and protective immunity.\(^{57,58}\) As briefly mentioned above, failed intrathymic deletion of “autoreactive” donor T cells can also contribute to cGVHD as evidenced by cGVHD induced by reconstitution of murine recipients with T-cell–depleted BM from allogeneic MHC class II–deficient donors that precludes thymic DC-mediated negative selection of maturing T cells.\(^{39}\) Intriguingly, thymectomy can prevent cGVHD pathology, suggesting that thymic dysfunction in cGVHD recipients favors selection of autoreactive and alloreactive T cells. Moreover, cGVHD and/or its therapy themselves are highly detrimental to thymic function.\(^{59}\) The possibility of shared mechanisms in the thymus and periphery is suggested by the finding of defective APC function in aGVHD mice.\(^{50}\) Collectively, these mechanisms can facilitate the emergence of self-reactive thymic emigrants and cGVHD induction caused by the de novo generation of both autoreactive and alloreactive donor CD4\(^+\) T cells as indicated by their capacity to induce cGVHD pathology upon their adoptive transfer in both syngeneic and allogeneic secondary recipients.\(^{24}\)

Conversely, keratinocyte growth factor administration, by reducing aGVHD-induced thymic injury, can improve thymopoiesis and restore thymic DC, resulting in amelioration of cGVHD.\(^{54}\) In summary, both mature T cells contained within the graft and precursor-derived thymic T cells mediate cGVHD pathology; however, their relative contribution to distinct cGVHD pathology and mechanism of action remain to be elucidated and is likely to vary between cGVHD models and between patients.

**T-cell effector mechanisms driving cGVHD pathology**

Conventional T cells can be broadly divided into Th1/Tc1 (interferon γ [IFNγ]), Th17/Tc17 (IL-17), and Th2 (IL-4/IL-10) subsets. Until recently, aGVHD was largely considered Th1-dominated, whereas cGVHD was considered to represent a Th2-mediated disease.\(^{12,61}\) This notion had its support in studies showing differential cytokine expression in aGVHD and cGVHD mice.\(^{62}\) Th2 cell accumulation in cGVHD mice,\(^{63}\) the relationship between G-PBSC, Th2/plasmacytoid dendritic cell (DC) skewing, and the higher incidence of cGVHD in patients.\(^{15,16,18}\) However, in both mice\(^{64}\) and humans,\(^{65}\) there is not a clear paradigm demonstrating that Th1 cells are required for aGVHD, whereas Th2 cells cause cGVHD.

Recently, the Th/Tc17 pathway has been shown to promote pathogenic autoimmune-mediated organ damage in multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and psoriasis.\(^{66}\) In systemic sclerosis, a condition closely resembling sclerodematous cGVHD, fibrosis, is mediated by T17 cells infiltrating the skin and serum IL-17 levels positively correlate with disease severity.\(^{67,68}\) Preclinical and clinical data support a role for IL-17 as a predictor and central mediator of pathology, especially the skin.\(^{28,53,70,71}\) In a preclinical study, high G-CSF doses were shown to invoke type 17 rather than type 1 or type 2 T-cell differentiation, and amplification of IL-17 production occurred in both CD4 and CD8 T cells.\(^{28}\) Donor IL-17A, predominantly Tc17 derived, promoted skin pathology (dermal thickening, loss of subcutaneous fat and hair follicles, and increased cellular infiltrate) and cutaneous fibrosis, manifesting as scleroderma, providing a logical explanation for the propensity of G-PBSCs to invoke sclerodematous cGVHD and highlighting Tc17 as an important cGVHD effector population. In clinical cGVHD studies, increased IL-17 messenger RNA transcripts and significant Tc17 infiltration were demonstrated in skin,\(^{72}\) whereas in the oral mucosa, Th17 infiltration dominated. Cytokines (IL-6, IL-21) known to support Th17 generation in GVHD are elevated in GVHD,\(^{73-76}\) and STAT3, which drives Th17 cellular plasticity.\(^{50,80-83}\) Just how these IL-17 inflammatory cytokines (eg, IL-22, IFNγ, granulocyte-macrophage colony-stimulating factor [GM-CSF]) and exhibit significant functional plasticity.\(^{50,80-83}\) Just how these IL-17–producing T cells generate fibrosis remains to be elucidated but some clear pathways have been highlighted and are outlined in the following list.

Burgeoning areas of investigation include analysis of:

1. TCR repertoires in the blood and organs of cGVHD mice and patients to determine whether there are dominant TCR clones that cause cGVHD;
2. Chemokine-facilitated migratory properties of T-effector cells in cGVHD\(^{64,85}\), and
3. The metabolic state of cGVHD T-effector cells that may suggest interventional approaches to prevent or treat cGVHD, as has been shown in preclinical aGVHD models.\(^{86-90}\)

**B cells and antibodies in cGVHD pathogenesis**

Emerging evidence supports an important role for donor B cells in both the initiation and perpetuation of cGVHD. In both mice and humans, B-cell homeostasis and tolerance mechanisms are disrupted after SCT, resulting in reduced memory B-cell formation and enrichment of activated transitional B cells in the reconstituting donor B-cell pool.\(^{91-93}\)
A correlation between reduced IL-10–producing Bregs (B10 cells) and cGVHD severity is increasingly reported. 96-98 B-cell–activating factor (BAFF), a cytokine critical for B-cell survival and maturation, is found in excess levels in patients with active cGVHD, resulting in increased BAFF-to-B-cell ratios. 98,99 In the setting of elevated BAFF levels, B cells reactive against polymorphic recipient (allo) or nonpolymorphic antigens shared by donor and host (auto)antigens, normally targeted for apoptotic death through negative selection, are protected and persist. Indeed, the association of BAFF and autoantibody production in cGVHD patients has been reported. 99 Recent preclinical studies in a multirgan nonsclerodermatous cGVHD model have demonstrated the requirement for increased T-follicular helper cells (Thf), germinal center (GC) B cells, and antibody which accumulates in target tissues, resulting in the development of some, although not all, manifestations of cGVHD. 100,101 Thf cells produce IL-21, a cytokine known to be critical for GC formation and the cutaneous and pulmonary manifestations of cGVHD. 28,29 Alloantibodies (predominantly to HY antigens) have been well described in cGVHD and correlate with disease activity. 17,48,102,103 Autoantibodies are widely detected in patients with cGVHD. Although initial reports suggested that antibodies to the platelet-derived growth factor receptor may be pathogenic, 104 this finding has been debated. 105 Mechanistically, the aberrant GC B-cell reaction seen in cGVHD results in antibody formation. 106 Elegant serum transfer experiments have now shown that antibody can be directly pathogenic and initiate disease. 106 Increasing BAFF concentrations have been associated with pre-GC B cells and post-GC plasma-like cells in patients, 107 which may be the result of either GC or extrafollicular B-cell responses, mechanisms yet to be determined in patients. Although peripheral blood Thf cell frequency has been reported to be reduced in patients with cGVHD, 108,109 Thf cells were skewed toward a highly activated profile with a predominance of Th2 and Th17 (IL-17, IL-21 producing) subsets, increased functional ability to promote B-cell immunoglobulin secretion and maturation, and an activation signature highly correlated with increased B-cell activation and plasmablast maturation. 108 Because in rodents GC B cells were quantified in the spleen, a plausible explanation for the reduced peripheral blood Thf frequency in cGVHD is that the Thf cells are localized in GCs within SLOs. However, cGVHD therapy or GVHD-induced injury to lymphoid organs resulting in decreased Thf production cannot be excluded. Consistent with that hypothesis, high plasma CXCL3 levels, which are chemottractant for T and B cells into SLOs, have been detected in cGVHD patients. 108 Because cGVHD is also characterized by autoantibody formation, it remains to be established whether the pathogenic antibodies in question are directed solely to allogeneic polymorphic antigens or also to nonpolymorphic “autologous” antigens shared by donor and recipient. Moreover, it is unclear whether antibody-dependent mechanisms are operative in all recipients with cGVHD, or only a subset; also unclear is the mechanism by which antibody initiates fibrosis and the cellular mediators involved remain to be elucidated.

**Role of macrophages in cGVHD pathogenesis**

Fibrotic injury is characterized by excessive accumulation of extracellular matrix (predominantly collagen) and fibroblasts, which replace parenchymal cells and impair normal tissue function. Macrophages play a crucial role in the tissue-repair response, are found in close proximity with collagen-producing fibroblasts and as demonstrated in multiple disease models, contribute to fibrosis. 100,111 In both preclinical and clinical cGVHD, macrophages have been shown to accumulate in fibrotic lesions. 28,72,112 However, the factors promoting macrophage tissue sequestration, and their mechanistic contribution to pathology have only recently been examined. In preclinical cGVHD models characterized by scleroderma or BO with multiorgan system fibrosis but without scleroderma, the sequestration of macrophages within skin and lung, and the subsequent development of cGVHD pathology, was shown to be both IL-17 and colony-stimulating factor 1 (CSF-1) dependent. 28,112 Tissue-infiltrating macrophages were of donor origin, alternatively activated (skewed toward anti-inflammatory responses) as indicated by their expression of CD206 rather than inducible NO synthase, and promoted pathology through their production of transforming growth factor β (TGFβ), a key cytokine for myofibroblast activation and collagen production. Importantly, the attenuation of CSF-1 receptor (CSF-1R) signaling using an anti-CSF-1R–blocking antibody depleted circulating and tissue-associated Ly6C60 monocytes, ablated tissue-infiltrating macrophages, and markedly attenuated both cutaneous and preexisting pulmonary cGVHD. 112 The mechanism by which IL-17 contributes to pathogenic macrophage migration and differentiation in cGVHD target organs remains undefined. However, IL-17 has been reported to function as a monocyte chemokine, to promote monocyte adhesion and elicit a proinflammatory transcriptome in macrophages, suggesting direct signaling of this lineage may be involved. 113 Other proinflammatory cytokines coproduced by Te17/Th17 such as GM-CSF may contribute synergistically to macrophage differentiation/polarization at localized sites.

Macrophages express very high levels of Fc-γ receptors and are highly efficient at opsonization of antibody-coated targets which in turn can generate very high levels of TGFβ. 114,115 Consistent with a link between antibody secretion and fibrosis, mice incapable of producing B cells or that produce B cells incapable of immunoglobulin isotype switching, 28 or that receive agents that either preclude GC formation 29,73,116 or deplete B cells 29,117,119 are unable to induce fibrosis or cGVHD. Thus, although unproven at this point, the interaction of allo-(and/or auto) antibody with tissue macrophages would appear an attractive unifying mechanism driving the aberrant macrophage differentiation and function that culminates in tissue fibrosis during cGVHD.

**Immune regulators of cGVHD**

Immune populations contained within the graft or that emerge from graft progeny can exhibit immune-modulatory capacity. 120 Tregs, defined by their coexpression of CD4, CD25, and the master transcription factor FoxP3, are critical for the control of innate and adaptive immune responses and can mediate tissue regeneration via amphiregulin release. 121 GC migratory Tregs, known as T-follicular regulatory cells, suppress GC responses. 122 Treg number or function perturbations lead to the development of autoimmune diseases and are thought to contribute to aGVHD and cGVHD pathology. 8,10,123 Both preclinical and clinical studies demonstrate that donor graft Treg number inversely correlates with aGVHD, 124-128 and cGVHD is associated with decreased numbers of circulating Tregs. 21,129,131 Factors contributing to diminished Treg numbers in cGVHD recipients remain to be fully elucidated although there are multiple candidates including diminished thymic production, reduced proliferative capacity of naïve Tregs, 132 and a failure in memory Treg survival due to their increased susceptibility to apoptosis. 131,133 DCs play an important role in the maintenance of Tregs in steady state and following SCT; 88,134,135 including cGVHD. 88,134,136 However, in recent
preclinical studies, donor DC MHC class II antigen presentation was shown to be impaired during aGVHD, and this resulted in a failure of Treg homeostasis that promoted cGVHD pathology.60,136

Although less well studied, altered Breg and NK development after SCT is thought to contribute to cGVHD. Breg function to suppress immune responses through multiple IL-10 and cell-cell contact-dependent mechanisms, including suppression of CD4 T-cell proliferation and IFNγ production, and monocyte TNF production.137,138

In patients with cGVHD, recent studies show that Breg numbers, including immunoglobulin M memory and transitional subsets, are reduced and exhibit a diminished capacity to produce IL-10.95,97 Enhanced NK reconstitution has also been shown to correlate with reduced incidence of cGVHD in the clinic, although not all studies show an inverse correlation between alloreactive NK cells and cGVHD.140 mechanistically, in preclinical studies, NK cells contribute to the regulation of CD4 and CD8 T-cell expansion through fas-mediated killing and competition for IL-15, respectively.141,142 Additionally, NK cells also produce cytokines that promote tissue regeneration, although whether this represents a functioning cGVHD mechanism remains to be investigated.143 Together, these studies highlight the potential clinical utility of therapeutic strategies, which promote the expansion of Bregs and NK cells after transplant.

**Figure 1. Schematic overview of the cellular and molecular mediators, known and implicated, contributing to the continuum of aGVHD and cGVHD pathology.**

Both naive T cells (Tna) and their precursors (HSCs, common lymphoid progenitor [CLP]) contained within the stem cell graft contribute to cGVHD pathology. Mature donor T cells within the graft contribute to thymic destruction resulting in disrupted immune reconstitution. Thymic dysfunction favors the selection of autoreactive and alloreactive T cells polarized toward Th17/Tc17 lineages. Donor-derived DC APC function is corrupted during aGVHD, reducing their capacity to expand and maintain Tregs in the periphery. T-follicular helper cell (TFH)-derived IL-21, together with elevated levels of BAFF, result in aberrant B-cell reconstitution favoring GC B-cell (GBC) expansion. Polyfunctional Th17/Tc17 cells migrate to target organs where secreted IL-17 may function as a chemokine for Ly6Clo monocytes. CSF-1 derived in part from Th17/Tc17 promotes the differentiation of Ly6Clo monocytes into tissue-resident macrophages (Mφ), which are polarized toward an M2 phenotype under the influence of multiple proinflammatory cytokines (GM-CSF, IL-22, IL-13, and IFNγ) produced by Th17/Tc17. Plasma cell-derived allo/autoantibodies (Ab) can bind to Fc receptors on macrophages, contributing to their polarization and promotion of TGFβ secretion, which promotes fibroblast activation and collagen production. Fc, receptor for immunoglobulins; T_{na}, alloreactive T cell; T_{eff}, effector T cell.

**New therapeutic strategies based on recent insights to pathophysiology**

Treatment of cGVHD is currently based on steroid administration and although many other approaches, including additional immune suppressants, UVB phototherapy, and extracorporeal photopheresis are commonly used, none have proven clearly effective.144,145 Thus, well-designed prospective studies based on NIH response criteria and our new understanding of cGVHD pathophysiology are needed. We now know that cGVHD develops via a complex cellular and molecular network involving thymic damage and aberrant antigen presentation leading to aberrant T- and B-cell reaction characterized by Th17/Tc17 differentiation, macrophage sequestration in tissue, alloantibody formation, and TGFβ-dependent fibrosis (Figure 1). Collectively, these studies highlight a number of therapeutic options. From a preventative aspect, the direct removal of naive αβ T cells from the graft (eg, using in vitro magnetic-based antibody approaches of T-cell removal or CD34+ stem cell selection)58,146 or depletion of differentiating T cells early after transplant (eg, by administering posttransplant cyclophosphamide to preferentially deplete alloreactive T cells while sparing Tregs)147 appears highly
effective at eliminating cGVHD. Approaches to inhibit the more terminal stages of aberrant (Th17/Tfh) T-cell development in cGVHD include small-molecule RORγt
t148 or STAT3 inhibitors and antibody-based therapeutics targeting IL-17 or IL-21 and their receptors.28,29

Strategies to enhance Treg numbers after SCT including Treg adoptive therapy to reconstitute the Treg pool have been adopted from rodent studies and are showing potential in the clinic.127,128,146,149-152

Recent preclinical studies show that Treg adoptive transfer can both prevent and treat cGVHD in mice with multiorgan system disease.136,153 Given the failure of Tregs during cGVHD and the challenges of generating sufficient Tregs for adoptive transfer to treat cGVHD patients, restorative approaches to date have focused on low-dose IL-2 administration to expand Tregs in vivo with ~50% of patients showing Treg expansion and some clinical response as long as therapy is continued.154,155 Recently, the adoptive transfer of Tregs with or without IL-2 and/or rapamycin has begun to be tested in clinical trials in an effort to increase the proportion and depth of patient response.

Approaches targeting B cells involve the prevention of aberrant B-cell development by administration of CD20 monoclonal antibody which appears effective in reducing disease severity in cGVHD patients when used as a preventative but not treatment strategy, likely due to the more effective B-cell depletion than that of antibody-secreting plasmablasts and plasma cells formed after cGVHD is established.156,157 Pursuing pharmacological agents that inhibit B- (with or without T-) cell activation, differentiation, and GC integrity by kinase inhibition (eg, Syk kinase, fostamatinib118; Bruton kinase; ibritumomab;117,118 Rho-associated kinase, KD025,73 and Janus kinase-1, ruxolitinib158) has a strong biological foundation, as confirmed in part by promising early clinical results already achieved with ruxolitinib159 and ibritumomab. At the most final stage of aberrant B-cell response, depletion of alloantibody-producing plasma cells by proteasome inhibition (eg, bortezomib) is supported by evidence of efficacy in animal systems and early clinical studies.160 Finally, targeting macrophages by preventing differentiation and survival in tissue through the inhibition of CSF-1R has proven highly effective in animal systems,112 as has the inhibition of TGFβ.112,161

References


Acknowledgments

The authors thank members of their laboratories, their collaborators, and the scientific community for providing the foundation for this review. The authors apologize to those investigators whose work they were unable to cite here. Lastly, the authors thank the patients who have participated in clinical studies that have fostered the advancement of the new therapies for this devastating disease.

This work was supported by grants from the Australian National Health and Medical Research Council (NH&MRC) APP1031728 (K.P.A.M.), National Institutes of Health, National Cancer Institute grants P01 CA142106-06A1 and P01 CA047741-20, National Institutes of Health, National Institute of Allergy and Infectious Diseases grants P01 AI056299 and R01 AI11879, and Leukemia & Lymphoma Society Translational Research grant 6458-15 and 6462-15 (B.R.B.). G.R.H. is an NH&MRC Senior Principal Research Fellow and Queensland Health Senior Clinical Research Fellow. K.P.A.M. is a Cancer Council Queensland Senior Research Fellow.

Authorship


Conflict-of-interest disclosure: The authors declare no competing financial interests.


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49. Coghill JM, Sarantopoulos S, Morani TP, Murphy WJ, Blazar BR, Sendroy JS. Effect of CD4+ T cells, the cytokines they generate, and GVHD on something old and something new. Blood. 2011;117(12):3268-3276.


111. Johnston HF, Xu Y, Racine JJ, et al. Administration of anti-CD20 Fab is highly effective in preventing but ineffective in treating chronic graft-versus-host disease while...


Chronic graft-versus-host disease: biological insights from preclinical and clinical studies

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