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

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Abstract

With the increasingly use of mismatched, unrelated and G-CSF 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 non-relapse mortality and morbidity. Chronic GVHD 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 a combination of mouse models and correlative clinical studies, has radically improved. We now understand that cGVHD is initiated by naïve T-cells, differentiating predominantly within highly inflammatory Th17/Tc17 and T-follicuar 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 (i.e. alloantibody) or non-polymorphic antigens common to both recipient and donor (i.e. autoantibody). It is now clear that alloantibody can, in concert with CSF-1-dependent donor macrophages, induce a TGFβ 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 naïve T-cell depletion, IL-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 eminent for this devastating transplant complication.

Introduction

Chronic graft-versus-host disease (cGVHD) remains the major cause of morbidity and non-relapse mortality after allogeneic hematopoietic stem cell transplantation (SCT).[1-3] Progress in improving cGVHD prevention and therapy have 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 or are supported by analysis of human materials acquired from cGVHD patients.

This review draws from animal model and clinical studies to provide an overview and the authors 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 of 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.

Since 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.[8-13] 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, non-conditioned murine cGVHD models will not be discussed in this review; instead we refer the reader to Chu et al.[9]

Chronic GVHD manifestations and initiating factors in the clinic

Chronic GVHD typically manifests with multi-organ 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 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 (i.e. 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) (e.g. macular-papular rashes, weight loss, diarrhoea 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 G-CSF mobilized peripheral blood stem cell grafts (G-PBSC) 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 to unmanipulated bone marrow (BM) grafts with the primary advantage of G-PBSC grafts is accelerated engraftment. A randomized trial of BM versus G-PBSC revealed similar overall survival with secondary endpoints showing that G-PBSC grafts provided decreased graft failure but with 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 accompany G-PBSC grafts. Risk factors for cGVHD development include preceding aGVHD, use of PBSC,[18] use of mismatched or unrelated donors (as opposed to matched siblings), transplant of female donors to male recipients, absence of ATG in conditioning and older recipients.[20] Given the expanding allo-SCT and G-PBSC graft use as well as the treatment of older recipients that historically were not candidates for allo-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 co-exist with or emerge from alloreactive T-cells.[22-24] Indeed, many aGVHD model systems have been adapted to infuse lower donor T cell numbers[25-27] 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 (e.g. at 4-8 weeks), the latter time favouring both escape from aGVHD lethality and a period of chronic TCR signalling.[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, GI 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[30]) or multi-organ 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 favour aGVHD. Since aGVHD can attack the thymus, BM and

secondary lymphoid organs (SLO), preceding aGVHD, even at a subclinical level in mice and patients, may have profound immunological manifestations resulting in T-cell or especially B-cell depletion[31-33] or loss of thymic function[34,35] that results in failed negative selection and loss of regulatory T-cell (Treg) production (see below).[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 is 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 HCT source and manipulations, if any. Other key variables may include mouse vendors[39] 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 mouse[42,43] 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 rodents[46] 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 employed for analysis of cGVHD pathogenesis. Rather we favour 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 versus cGVHD independent of the type (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 co-ordinated 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 characterised by pro-inflammatory cytokine release (e.g TNF, 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 that receive the transplantation procedure. These cytokines, together with luminal damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) released from damaged gut tissue and the microbial luminal contents, results in the activation of antigen presenting cells (APC). Activated APC then prime naïve donor T-cells and preferentially drive Th1/Tc1 and Th17/Tc17 differentiation and expand Teffector cells, which can mediate target tissue GVHD, including the thymus and SLO, as well as the skin, liver, GI 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 sclerodermatous cGVHD.[28,53] While donor natural killer (NK) cells, Tregs regulatory B-cells (Breg) and macrophages play important roles in dampening both aGVHD and cGVHD (see below), 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 below). 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 graft T-cell compartment is comprised of antigen-inexperienced naïve and antigen-experienced Teffector and memory subsets. In both pre-clinical 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.[38] Intriguingly, thymectomy can prevent cGVHD pathology, suggesting that thymic dysfunction in cGVHD recipients favours selection of auto- and allo- reactive 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.[60] Collectively these mechanisms can facilitate the emergence of

self-reactive thymic emigrants and cGVHD induction caused by the de novo generation of both auto- and allo-reactive 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.[24] 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 (IFNγ), Th17/Tc17 (IL-17) and Th2 (IL-4/IL-10) subsets. Until recently, aGVHD was largely considered Th1-dominated, while 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 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, while Th2 cells cause cGVHD.

Recently the Th/Tc-17 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 sclerodermatous cGVHD, fibrosis is mediated by Th17 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[69] 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-PBSC to invoke sclerodermatous cGVHD and highlighting Tc17 as an important cGVHD effector population. In clinical cGVHD studies, increased IL-17 mRNA transcripts and significant Tc17 infiltration were demonstrated in skin,[72] while 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, that drives Th17 development and Th17-dependent autoimmunity,[77] is essential for CD4+ T-cell-mediated sclerodermatous cGVHD.[78] Lichenoid cGVHD in patients have co-existing Th1 and Th17 cells with increased CD8+ T cells producing IL-17 and IFNγ[72,79] and IL-17 is systemically elevated late after SCT as cGVHD develops.[76] In multiple disease models including GVHD, both Th17 and Tc17 cells co-express multiple pro-inflammatory cytokines (e.g. IL-22, IFNγ, 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 below.

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 Teffector cells in cGVHD;[84,85] and 3. the metabolic state of cGVHD Teffector 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 regulatory B cells (Breg or B10 cells[94]) and cGVHD severity is increasingly reported.[95-97] B cell activating factor (BAFF), a cytokine critical for the B-cell survival and maturation, is found in excess levels in patients with active cGVHD, resulting in increased BAFF/B cell ratios.[98,99]. In the setting of elevated BAFF levels, B-cells reactive against polymorphic recipient (allo) or non-polymorphic 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 pre-clinical studies in a multi-organ non-sclerodermatous cGVHD model have demonstrated the requirement for increased T follicular helper cells (TFh), germinal center (GC) B cells and antibody which accumulates in target tissues, resulting in the development of some, although not all, manifestations of cGVHD.[26,29,100,101] Tfh 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 antigen) have been well described in cGVHD and correlate with disease activity.[47,48,102,103] Autoantibodies are widely detected in patients with cGVHD. While initial reports suggested antibodies to the PDGFR may be pathogenic,[104] this finding has been debated.[105] Mechanistically, the aberrant GC B-cell reaction seen in cGVHD results in antibody formation.[29] 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 extra-follicular B cell responses, mechanisms yet to be determined in patients. While peripheral blood (PB) Tfh cell frequency has been reported to be reduced in patients with cGVHD,[108,109] Tfh cells were skewed toward a highly activated profile with 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] Since in rodents, GC B-cells were quantified in the spleen, a plausible explanation for the reduced PB Tfh frequency in cGVHD is that the Tfh cells are localized in GCs within SLO. However, cGVHD therapy or GVHD-induced injury to lymphoid organs resulting in decreased Tfh production cannot be excluded. Consistent with that hypothesis, high plasma CXCL3 levels, which are chemoattractant for T- and B-cells into SLO, have been detected in cGVHD patients.[108] Since cGVHD is also characterized by autoantibody formation, it remains to be established whether the pathogeneic antibodies in question are directed solely to allogeneic polymorphic antigens or also to non-polymorphic "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.[110,111] 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 multi-organ 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 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 iNOS, and promoted pathology through their production of TGFβ a key cytokine for myofibroblast activation and collagen production. Importantly, the attenuation of CSF-1R signalling using an anti-CSF-1R blocking antibody depleted circulating and tissue associated Ly6C^{lo} monocytes, ablated tissue infiltrating macrophages and markedly attenuated both cutaneous and pre-existing 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 signalling of this lineage may be involved.[113]. Other pro-inflammatory cytokines co-produced by Tc17/Th17 such as GM-CSF[50] may contribute synergistically to macrophage differentiation/polarization at localized sites.

Macrophages express very high levels of Fc-gamma receptors and are highly efficient at opsonisation 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 IgG isotype switching,[26] or who

recieve agents that either preclude GC formation[29,73,116] or deplete B cells[29,117-119] are unable to induce fibrosis or cGVHD. Thus, whilst 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] Treg, defined by their co-expression 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 Tfollicular regulatory cells, suppress GC responses.[122] Treg number or function perturbations lead to the development of autoimmune diseases and are thought to contribute to acute and chronic GVHD 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 Treg.[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 Treg[132] and a failure in memory Treg survival due to their increased susceptibility to apoptosis.[131,133] DC play an important role in the maintenance of Treg 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-dependant 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 IgM 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 clinical setting,[139] although not all studies show an inverse correlation between alloreactive NK cells and cGVHD.[140] Mechanistically, in preclinical studies, NK 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 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 Breg and NK after transplant.

New therapeutic strategies based on recent insights to pathophysiology

Treatment of cGVHD is currently based on steroid administration and while many other approaches, including additional immune suppressants, UVB phototherapy and extracorporeal photophoresis are commonly employed, 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 (Fig 1). Collectively, these studies highlight a number of

therapeutic options. From a preventative aspect, the direct removal of naïve αβ T cells from the graft (e.g. 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 (e.g. by administering post-transplant cyclophosphamide to preferentially deplete alloreactive T-cells whilst sparing Treg)[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[148] 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 wth multi-organ system disease.[136,153] Given the failure of Treg during cGVHD and the challenges of generating sufficient Treg for adoptive transfer to treat cGVHD patients, restorative approaches to date have focused on low dose IL-2 administration to expand Treg in vivo with approximately 50% of patients showing Treg expansion and some clinical response as long as therapy is continued.[154,155] Recently the adoptive transfer of Treg 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 responds.

Approaches targeting B-cells involve the prevention of aberrant B cell development by administration of CD20 mAb that appear 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 (e.g.

Syk kinase, Fostamatinib;[118] Bruton kinase; Ibrutinib;[117] Rho-associated kinase, KD025,[73] and Janus Kinase-1, Ruxolitinib[158]) have a strong biological foundation, as confirmed in part by promising early clinical results already achieved with Ruxolitinib[159] and Ibrutinib. At the most final stage of aberrant B cell response, depletion of alloantibody-producing plasma cells by proteosome inhibition (e.g. 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 the CSF-1 receptor has proven highly effective in animal systems,[112] as has the inhibition of TGFβ.[112,161]

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KPAM, GRH, and BRB wrote the paper.

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Authors state no conflict of interest related to the above work.

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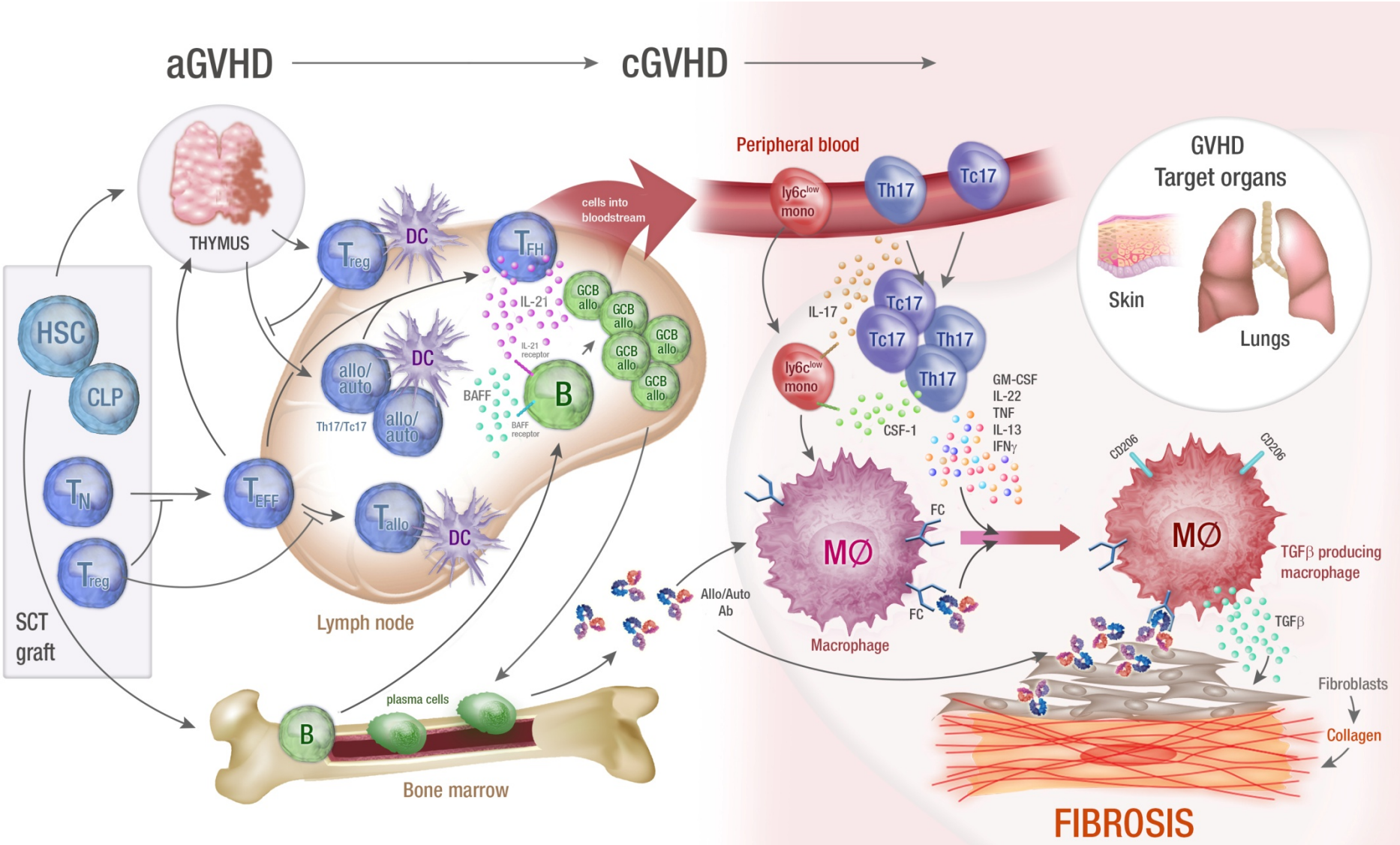
Figure Legend

Figure 1. *Schematic overview of the cellular and molecular mediators, known and implicated, contributing to the continuum of acute and chronic GVHD pathology*. Both naïve T cells (T_N) and their precursors (HSC, 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 favours the selection of auto and alloreactive T cells polarized toward Th17/Tc17 lineages. Donor derived dendritic cells (DC) antigen presenting cell function is corrupted during aGVHD, reducing their capacity to expand and maintain Treg in the periphery. T follicular helper cell (T_{FH}) derived IL-21, together with elevated levels of BAFF result in aberrant B cell reconstitution favouring germinal centre 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 macropahges, which are polarized toward an M2 phenotype under the influence of multiple proinflammatory cytokines (GM-CSF, IL-22, IL-13 and IFNg) produced by Th17/Tc17. Plasma cell derived allo/auto antibodies (Ab) can bind to Fc receptors on macrophages, contributing to their polarization and promotion of TGFβ secretion, which promotes fibroblast activation and collagen production.


aGVHD
cGVHD
THYMUS
HSC
CLP
T_N
T_{reg}
SCT graft
T_{EFF}
T_{reg}
DC
T_{FH}
IL-21
IL-21 receptor
GCB allo
allo/ auto
Th17/Tc17
BAFF
BAFF receptor
B
T_{allo}
Lymph node
cells into bloodstream
plasma cells
B
Bone marrow
Allo/Auto Ab
Peripheral blood
ly6c^{low} mono
Th17
Tc17
IL-17
CSF-1
GM-CSF
IL-22
TNF
IL-13
IFNγ
MØ
FC
Macrophage
CD206
TGFβ producing macrophage
TGFβ
Fibroblasts
Collagen
FIBROSIS
GVHD Target organs
Skin
Lungs

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