Review

Targeting the IL17 Pathway for the Prevention of Graft-Versus-Host Disease

CrossMark

ASBMT
American Society for Blood and Marrow Transplantation

Anniek B. van der Waart [1,2], Walter J.F.M. van der Velden [3,4],
Nicole M. Blijlevens [3], Harry Dolstra [1,2,*]

[1] Department of Laboratory Medicine - Laboratory of Hematology, Radboud University Medical Center, Nijmegen, The Netherlands
[2] Nijmegen Centre for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands
[3] Department of Hematology, Radboud University Medical Center, Nijmegen, The Netherlands
[4] Nijmegen Institute for Infection, Inflammation and Immunity, Radboud University Medical Center, Nijmegen, The Netherlands

A B S T R A C T

Graft-versus-host disease (GVHD) is still a major complication of allogeneic stem cell transplantation (allo-SCT). The pathophysiology of GVHD is a multistep process initiated by tissue damage and proinflammatory cytokine cascades induced by the pretransplantation conditioning therapy. This eventually results in Th1-driven tissue damage. However, increasing evidence indicates the involvement of IL17-producing T cells in GVHD pathogenesis. Both CD4$^+$ and CD8$^+$ IL17-producing T cells are suspected of initiating the Th1 response and aggravating tissue inflammation, resulting in full-blown GVHD. In this review, we discuss the involvement of IL17-producing T cells in GVHD and the factors involved in their expansion, differentiation, and activation. Different dendritic cell (DC) subsets, such as plasmacytoid DCs and DC NK lectin group receptor 1$^+$ myeloid DCs have the capability to stimulate Th/Tc17 responses through the release of cytokines. Pivotal cytokines include IL1β, IL6, IL23, and TGFβ, which are known to drive differentiation and expansion of IL17-producing T cells, and these cytokines are highly elevated in patients after allo-SCT. Potent activators of these DC subsets are motifs that are released upon tissue damage and microbial exposure during allo-SCT. These motifs aggravate the Th/Tc17 response via the activation of various pathogen recognition receptors, thereby initiating and perpetuating GVHD. A more comprehensive understanding of the factors and DC subsets driving the IL17 pathway will result in developing and testing novel therapeutic approaches for the prevention of GVHD.

© 2014 American Society for Blood and Marrow Transplantation.

INTRODUCTION

Graft-versus-host disease (GVHD) is still a major complication and limitation of allogeneic stem cell transplantation (allo-SCT) [1,2]. The pathophysiology of GVHD is a multistep process involving tissue damage and proinflammatory cytokine cascades induced by the pretransplantation conditioning therapy [2,3]. Furthermore, innate immune activation caused by perturbed host-microbe interactions at epithelial barriers increases inflammation and antigen presentation [4]. This results in an excessive inflammatory environment in which donor-derived CD4$^+$ and CD8$^+$ T cells become activated by antigen-presenting cells. In addition, T cell trafficking towards inflamed GVHD-prone organs, such as skin, lung, the gastrointestinal tract, and liver, is strongly augmented. Further, tissue destruction in these organs occurs in the case of presentation of ubiquitous or epithelial expressed allo-antigens to infiltrating alloreactive T cells. Moreover, dendritic cells (DC), macrophages, and other T cell subsets are recruited, resulting in further enhancement of GVHD. In particular, T helper (Th) 1-type CD4$^+$ T cells and T cytotoxic (Tc)-1 type CD8$^+$ T cells play an important role in the effector phase of GVHD pathophysiology [1,2,5]. However, recent studies indicate that other T cell subsets, such as Th/Tc17 cells, are involved in the initiation and aggravation of GVHD [6–9]. In this review, we will discuss the role of these IL17-producing T cells in GVHD development and the potential therapeutic strategies targeting the IL17 pathway to diminish or prevent this detrimental complication.

## REQUIREMENTS FOR DIFFERENTIATION OF IL17-PRODUCING CELLS

Generally, IL17-producing T cells differentiate from naïve T cells, though plasticity of other T cell subsets into IL17-secreting T cells has been shown [10]. Activation of the transcription factor retinoic acid-related orphan receptor (ROR)γt is essential for IL17-producing CD4$^+$ (Th17) and CD8$^+$ (Tc17) T cells [11] that can be achieved via various routes, though it generally occurs via the STAT3 signaling pathway. STAT3 is not only responsible for the activation of RORγt, but it can also bind and function as a promoter to other genes involved in this process, such as IL17 [12].

Various cytokines play a major role in the activation of STAT3, RORγt, and Th/Tc17 differentiation (Figure 1). The importance of IL1β and IL6 has been shown in several studies, both in mouse models as well as in vitro cultures of human T cells [13,14]. The additional role of TGFβ in this process is still unclear, mainly due to differences in mouse and human studies. Most mouse studies showed that TGFβ is essential in Th/Tc17 differentiation. However, human Th/Tc17 cells can be generated in vitro without the presence of TGFβ [13].
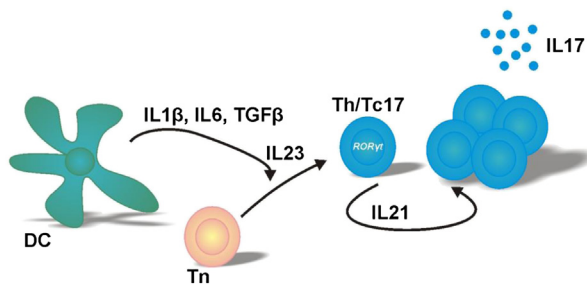
Figure 1. Th/Tc17 cell differentiation. Cytokines produced by dendritic cells differentiate naive T cells (Tn) towards Th/Tc17 cells. DC indicates dendritic cell; Tn, naive T cell; Th/Tc, helper T cell/cytotoxic T cell.

Additional studies in humanized mouse models could give more insight in this process. Generally, in the presence of IL1β and IL6 (and possibly TGFβ), while IFNγ and IL4 are absent to induce Th1 or Th2 skewing, differentiation to IL17-producing T cells is initiated. In addition, IL23 plays a pivotal role in the commitment and maintenance of Th17 cells [14].

Furthermore, IL23 was shown to play a major role in the generation of pathogenic Tc17 cells, as IL23-treated Tc17 cells were potent inducers of autoimmune diabetes, whereas Tc17 cells treated with TGFβ and IL6 were not [15]. Finally, the cytokine IL21 is also involved in Th17 differentiation [16]. Upon initial activation of STAT3 by IL6, both IL21 and IL23, along with TGFβ, induced IL17 expression via the activation RORγt. Moreover, the cytokine IL21 is also involved in self-maintenance, as this cytokine is produced by the Th/Tc17 cells themselves.

Multiple microenvironmental factors could also influence Th17 differentiation. For instance, the presence of retinoic acid in the microenvironment inhibits Th17 differentiation and induces regulatory T cell (Treg) formation [17]. On the other hand, several labs have described a role for the aryl hydrocarbon receptor in Th17 differentiation [18]. These studies showed that aryl hydrocarbon receptor ligands increase Th17 responses from naïve T cells and promote autoimmunity in mouse models. The various cytokines required for Th/Tc17 differentiation are released from diverse immune (eg, myeloid DC [mDC] and plasmacytoid DC [pDC]) and nonimmune antigen-presenting cells on activation of pathogen recognition receptors (PRRs) by motifs released upon tissue damage and/or microbial exposure, including various danger-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), respectively. Because in the human body epithelial barriers are inhabited by multiple microbes, both Th17 and Tc17 cells are of particular importance in the protection against extracellular microbes and defense mechanisms in the epithelial barriers of skin, gut, and lung. The release of IL17 has, indeed, been shown to enhance barrier defense, for example, by the production of antimicrobial peptides. Consistently, Th/Tc17 cells have been described to play a major role in the initiation of autoimmune disorders and other inflammatory conditions that originate at epithelial barriers, such as Crohn's disease and psoriasis [19]. As GVHD predominantly affects the gut, skin, and liver, Th/Tc17 cells are also suspected to be major players in the onset of GVHD.

TH/TC17 CELLS AND THEIR ROLE IN GVHD
Indicative Data from Animal Models

Several mouse studies demonstrated the involvement of Th/Tc17 cells in the onset and persistence of GVHD [7,20,21].

Carlson et al. showed development of lethal GVHD upon injection of in vitro–generated Th17 cells in a bone marrow transplantation mouse model [7]. In addition, adoptive transfer of IL17$^{-/-}$ T cells delayed the occurrence of GVHD compared with wild type (WT) T cells [21]. However, the precise role of Th/Tc17 cells in GVHD is still debated. For instance, Yi et al. reported initiation of Th1-driven GVHD in the absence of IL17 [22]. To the contrary, Yi et al. also observed IL17–driven GVHD when the Th/Tc1 cytokine IFNγ was absent [23]. A joint event was described by Yu et al., who observed lower GVHD incidence while sparing the graft-versus-tumor (GVT) effect, but only when both the Th/Tc17 driving transcription factor RORγt as well as Th/Tc1–inducing transcription factor t-bet were targeted simultaneously [24]. Targeting only RORγt elevated IFNγ levels, whereas IL17 was highly increased when t-bet was abolished. However, targeting both resulted in the lack of both cytokines and the absence of GVHD.

Alternatively, the observed effect on GVHD could also be explained by increased levels of Tregs in these mice. Interestingly, Fulton et al. attenuated GVHD by only targeting RORγt, though in this mouse model no increase in IFNγ was observed as a consequence of lacking IL17 [25]. In addition, targeting RORγt in this mouse model only affected serum IL17, whereas Yu et al. showed disturbed levels in several GVHD target organs [24,25]. Taking into account the differences in human and mouse Th/Tc17 differentiation, more insight in this process could be revealed in humanized mouse models.

In addition to IL17, Th/Tc17 cells could also play a role in GVHD via production of IL22 [26]. IL22, which is related to the IL10-family, has a paradoxical role in inflammation with both protective and inflammatory effects. Though it was shown that IL22 produced by innate lymphoid cells protected intestinal stem cells from immune-mediated damage during GVHD [27], IL22 is also involved in the pathogenesis of several inflammatory diseases, such as rheumatoid arthritis and psoriasis [28,29]. Interestingly, a recent study revealed a possible role of IL22 in GVHD, as adoptive transfer of IL22$^{-/-}$ instead of WT T cells reduced acute GVHD severity [30]. However, IL17 production of IL22$^{-/-}$ T cells was not affected, and an increase in Tregs was observed in these mice, which could also contribute to the reduced GVHD. In conclusion, collective findings in experimental mouse models suggests that Th/Tc17 could contribute to the pathophysiology of GVHD through IL17, and possibly IL22, production.

Studies in Allo-SCT Patients

In addition to their role in mice, a key role of Th/Tc17 cells in human GVHD pathogenesis has been reported [6,8]. Interestingly, increased levels of circulating Th17 cells have been described in GVHD patients [8,31]. For instance, Liu et al. showed increased circulating IL17-producing CD4$^+$ T cells in patients at the onset of GVHD, compared with time-matched controls [31]. Furthermore, a single nucleotide polymorphism (SNP) in the IL23 receptor, important in Th/Tc17 differentiation, has shown a protective role in the development of acute GVHD in 2 independent studies [32,33]. However, as the functional consequence of the SNP is yet unknown, it cannot be stated whether IL17 differentiation would be inhibited or enhanced. Although the potential involvement of Th17 cells in GVHD has been proposed by multiple studies, Broady et al. found an expansion of Th1, rather than of Th17 cells, in GVHD-affected skin [34].

However, Th17 cells have been shown to coproduce IFNγ or revert completely into Th1 cells [35]. Regarding this proposed plasticity of Th17 responses, other (surface) markers would be more reliable to study the involvement IL17-producing T cells in GVHD. Using CD161, the distinguishing surface marker for both CD4$^+$ and CD8$^+$ T cells capable of producing IL17 [36], we recently showed decreased levels of circulating Th/Tc17 cells in GVHD patients, although they infiltrated GVHD-affected skin and gut [9]. This was in concordance with data published by Bossard et al., who reported significantly higher absolute numbers of Th/Tc17 cells in GVHD-affected intestinal mucosa, using the markers CD161, RORγt, and CC chemokine receptor (CCR)6 [6]. This suggests that Th/Tc17 cells could be involved in the onset of GVHD via their migration potential toward GVHD target organs, thereby exerting a proinflammatory effect, as well as increasing the recruitment of alloreactive Th/Tc1 cells and other immune cells. However, many factors, such as the conditioning regimen and different post-transplantation immune suppressive therapy, will have a prominent impact on expansion kinetics and involvement of Th/Tc17 and Th/Tc1 responses in GVHD in patients.

TH/TC17 TRAFFICKING TO GVHD TARGET ORGANS

Migration towards GVHD target organs could take place via multiple chemokine axes, as Th/Tc17 cells display high levels of the chemokine receptors CCR6 and CXCR3 [26]. Interestingly, we showed that a SNP in CCR6 present in the donor resulted in lower GVHD incidence [37]. Its ligand, CCL20, is constitutively expressed in the GVHD target organs, such as the liver, colon, small intestine, lung, and skin [38].

Additionally, damage to the epidermal permeability barrier, as well as stimulation with IL1β—both known to be elevated after conditioning of allo-SCT patients—results in upregulation of CCL20 expression [9,39,40]. We, indeed, showed that GVHD-affected skin and gut display high expression of CCL20, both by keratinocytes and infiltrating immune cells [9]. Furthermore, increased expression of the CXCR3-ligands, CXCL9 and CXCL10, has also been described in serum of GVHD patients and in their affected tissues [41]. These chemokines, which are strongly upregulated upon inflammation, can attract migrating Th/Tc17 cells, resulting in their recruitment into GVHD-affected tissues, and therefore decreased levels of circulating CXCR3$^+$ T cells as described in GVHD patients [41,42]. This endorses the various migration routes of Th/Tc17 cells towards GVHD-target tissues.

CROSSTALK OF TH/TC17 WITH TH/TC1 CELLS

Upon recruitment of Th/Tc17 cells into GVHD tissues, other T cell subsets are attracted. Crosstalk between Th/Tc1 and Th/Tc17 cells in GVHD development is very likely, as has also been suggested in other immune-related diseases [43,44]. In this regard, we suggest that shortly after allo-SCT, Th/Tc17 cells could play an important role in initiating the GVHD response, which is thereafter overshadowed by a tremendous Th/Tc1 response, causing the main damage in the tissues. This hypothesis has been underscored by histological data showing that in early-stage mild gut GVHD, higher numbers of Th17 cells were found than in patients with severe GVHD after allo-SCT [45]. Possibly, Th/Tc17 and Th/Tc1 responses act either sequentially or simultaneously together in GVHD. This was also observed in inflammatory bowel disease, another IL17-driven disease, in which a synergy of Th1 and Th17 cytokines was observed [43].

The dependence of these subsets upon each other has been clearly demonstrated in different studies. In the absence of IFNγ (with or without IL4), differentiation towards IL17-producing T cells was increased, resulting in augmented GVHD [23]. However, visa versa, lack of IL17 increased IFNγ production, resulting in Th1-driven GVHD [22]. Taken together, crosstalk between Th/Tc17 and Th/Tc1 cells is apparent in the setting of GVHD, in which upon initiation of Th/T17 cells, Th/Tc1 cells will be potently activated and aggravate the disease. However, no hard evidence yet exist for this sequential T cell response and further research is warranted to understand dynamics and crosstalk of these 2 cooperating T cell subsets in GVHD.

FACTORS PROMOTING TH/TC17 ACTIVATION AND EXPANSION AFTER ALLO-SCT
Role of DC Subsets

The role of DCs in GVHD development was established more than a decade ago by Shlomchik et al. [46]. Although it has been demonstrated that both DCs from the host as well as from the donor can contribute, less is known about the role of the different DC subsets in GVHD. The most common known DCs, the mDCs, have been shown to play a role in GVHD in several mouse models [46,47]. Especially, depletion of host mDCs in recipient mice has been shown to prevent GVHD. In addition, low levels of circulating mDCs in allo-SCT patients have been described to correlate with the development of severe acute GVHD, suggesting migration of these cells to GVHD sites [48].

Another DC subset, which has recently been associated with GVHD, are pDCs. Notably, increased levels of pDCs were detected in affected gut tissue of acute GVHD patients [6]. Also, a decrease in circulating pDCs was correlated with acute GVHD, suggesting active migration from the periphery into inflamed tissue [48]. Furthermore, pDCs are capable of producing high levels of IL1β, a key cytokine in the development of GVHD and the IL17 differentiation pathway [49,50]. Interestingly, it was shown that presence of IL1β resulted in increased IL17 production by CD161-expressing CD8$^+$ Tc17 cells [51]. Moreover, pDC leukemia is often associated with isolated cutaneous lesions due to skin accumulation of leukemic pDCs [52], suggesting their involvement in inflammatory diseases of the skin, such as GVHD.

Moreover, pDCs could be potentially activated via TLR9 by self-DNA, released upon pretransplantation conditioning. Although viral DNA can gain access to the intracellular TRL9 receptor, self-DNA does not gain direct entry to endosomes and TLR9 under normal conditions. However, in the presence of the endogenous cationic antimicrobial peptide LL37, pDCs can be also activated by self-DNA [53]. The main function of LL37 peptide is to prevent microbial invasion of damaged epithelial surfaces by directly killing a broad spectrum of pathogens, including bacteria, protozoa, fungi, and viruses. Besides this, it has the ability to bind self-DNA, released by, for instance, dying cells after the conditioning regimen, to form aggregates and condensed particles that are transported into pDCs, allowing TLR9 signaling [53,54]. LL37 is normally not expressed in healthy skin, but it is transiently produced by keratinocytes and released by infiltrating neutrophils in response to skin wounds or infections [55]. In psoriatic skin, LL37 has been identified as a key mediator in pDC activation [53]. As the conditioning regimen, containing chemotherapy and total body irradiation, probably results in increased LL37 and self-DNA exposure, and thereby robust

pDC activation, a Th/Tc17 response will be triggered, starting the inflammatory response of GVHD.

Another relevant DC subset possibly involved in the initiation of GVHD could be DC NK lectin group receptor 1 (DNGR1)$^+$ CD141$^+$ mDCs. This DC subset, capable of producing IL6, IL12, TNF, and IFN, has the capacity to induce Th17 responses [56,57]. Recently, it was shown that these DNGR1$^+$ DCs are activated upon interaction with filament actin, which has been shown to be strongly exposed upon cell damage [58]. Because the pretransplantation conditioning regimen of allo-SCT patients results in host tissue damage and, thereby, possible exposure of filament actin, DNGR1$^+$ DCs could be activated and, thereby, promote IL17 responses.

Therefore, both pDCs as well as DNGR1$^+$ DCs could play a key role in the activation of IL17-producing T cells and, thereby, the onset of GVHD.

Role of Microbe and Damage Association Patterns

The role of innate immune activation by host microbe interactions at epithelial barriers in the pathogenesis of GVHD is increasingly recognized [4,59,60]. In 1971, it was shown by studies in chimeric mouse models that germ-free or gut-decontaminated mice showed reduced GVHD-related mortality [61,62]. Recently, the composition of the gut microbiota and changes that occurred therein after allo-SCT were shown to be pivotal in the pathogenesis of GVHD in both mice and humans, supporting previous observations [63].

Bacteria and fungi, and also viruses, have been associated with the occurrence of GVHD [2,64,65]. In addition, the use of antibiotics resulted in reduced anaerobic bacteria and a lower incidence of acute GVHD [66]. The fungus Candida albicans has also been shown to result in increased incidence of acute GVHD [67]. This was in line with the study of Marr et al. who showed that prophylactic fluconazole use prevented Candida infections and resulted in a lower GVHD incidence [68].

These microbes and their PAMPs, that potentially contribute to GVHD, are mainly present early after allo-SCT, which is during the initiation phase of GVHD [2]. Generally, DC activation by microbes can induce Th1, Th2, and/or Th17 responses, dependent on the type of antigen. However, most of these commensal microbes and pathogens can elicit strong Th17 responses. As several microbes elicit plastic immune responses during the progress from mucosal to systemic infection, with Th17 responses preceding Th1, it can be imagined that Th/Tc17 might be an early event in GVHD that precedes massive Th/Tc1−driven tissue damage. Microbes could influence Th17-mediated GVHD indirectly by activating innate immune cells via PRRs and, more directly and specifically, by eliciting CD4$^+$ and CD8$^+$ T cell responses through the several DCs subsets. The role of the latter in GVHD is unknown, but it is suggested by a role for microbe-specific CD4$^+$ Th17 and Th1 responses in colitis and syngeneic GVHD of the gastrointestinal tract [69,70]. In addition, Jankovic et al. showed that activation of the PRR NLRP3 by PAMPs and DAMPs, ie, uric acid, contributed significantly to the occurrence of intestinal GVHD [71]. The observation that this was mediated by IL1β, followed by a Th17 response especially indicates the role of Th17 cell as early reactor after activation signals.

Furthermore, it has been observed that Th17 cells respond to several microbial antigens [72,73]. More specifically, CD161-expressing T cells can be activated by Escherichia coli resulting in an inflammatory cytokine response [72]. In addition, IL17 secretion by CD4$^+$ T cells was observed upon stimulation with Candida albicans [73]. Plausibly, pDCs could play a role in the activation of Th17 cells by microbes via TLR 7 and 9 [74]. In this regard, Yu et al. found increased IL17 production by T cells upon coculture with TLR7 stimulated pDCs [50]. In addition, similar data were observed when pDCs were activated with Influenza A or HIV-infected cells. Also, as viral infections by herpes simplex virus and cytomegalovirus have been associated with the occurrence of GVHD, activation of pDCs by these viruses could contribute to the initiation of the disease [64,65]. Furthermore, pDCs are activated by extracellular and intracellular bacterial DNA and RNA, supposedly via TLR7 and TLR9 after CD32-mediated uptake [75,76]. In an animal model for the Th17-driven disease multiple sclerosis, initiated by exposure to Mycobacterium tuberculosis and pertussis toxin, depletion of pDCs resulted in less severe clinical symptoms as well as less Th17 cells [49]. All together, this supports the notion that pDCs can initiate a Th/Tc17 response via activation by microbial antigens.

Role of Cytokines

Pretransplantation conditioning regimen results in tissue damage with a subsequent rise of various (proinflammatory) cytokines [77,78]. Within the first weeks after allo-SCT, levels of IL1β, IL6, IL8, IL10, IL21, IL23, and TNFα are increased, which can remain high up to 12 weeks after allo-SCT [31,77]. Increased levels of IL6 have been reported in several studies and have been correlated with the occurrence of infections and GVHD [79,80]. Importantly, TGFβ appears also elevated during the first weeks after allo-SCT in patients with acute GVHD [78]. As described in previous paragraphs, these cytokines are mainly produced by DCs upon activation due to tissue damage and microbe exposure. Several of these cytokines are involved in the differentiation and expansion of Th/Tc17 cells, which could result in increased levels of Th/Tc17 cells after allo-SCT, as previously described by us and others [8,9].

Additionally, a trend towards higher IL17 serum levels has been observed in patients with acute GVHD [81]. This is in alliance with data published by Espinoza et al., who found that patients receiving a transplant from donors with a SNP in the promoter region of the IL17 gene were more prone to developing GVHD [82]. Interestingly, T cells with this allele produced more IL17 than those without. Furthermore, Lui et al. showed increased IL17 in the serum of GVHD patients; however, IL22, also produced by Th/Tc17 cells, was decreased in these patients [31].

In addition to IL17 and IL22, Th/Tc17 cells also produce IL21 to support self-renewal [16]. The lack of IL21R on T cells prevented GVHD while sparing the GVT effect [83]. Moreover, antibody blockade of IL21 also protected mice from the development of GVHD [84,85]. However, 2 out of these 3 models showed minimal responsive IL17 levels, and the observed effect could also be explained by the increased levels of Tregs [83,85]. Nevertheless, Hippen et al. showed decreased levels of IL17 upon blockade of IL21, although also in this study an increase of Tregs was observed [84]. In addition, given the importance of IL21 in T cell proliferation, blockade of IL21 also resulted in decreased numbers of alloreactive T cells, which could also contribute to the reduced GVHD response in this model. Taken together, the increased levels of cytokines induced by the conditioning regimen results in expansion and activation of Th/Tc17 cells after allo-SCT. This could be an important, initial driver of the development of GVHD, though the cytokines produced by the Th/Tc17 cells might not all contribute to GVHD development.

TARGETING TH/TC17 RESPONSES TO PREVENT GVHD

Currently, GVHD treatment options rely largely on the suppression of the whole immune system, using immunosuppressive drugs such as cyclosporine A and mycophenolate mofetil. Though these treatments can reduce GVHD intensity in a proportion of the patients, treatment success remains modest, especially in patients with severe GVHD [86]. In addition, suppression of the whole immune system predisposes patients to an increased risk of infections and tumor relapse. Therefore, selective prevention of GVHD would be a far more favorable strategy. With more comprehensive understanding of which factors and T cells subsets are involved in GVHD development, improved targeting strategies could be exploited to prevent GVHD. Selective GVHD prevention will likely reduce the nonrelapse-related mortality, and, importantly, increase quality of life.

Manipulation of T Cells in Graft and Donor Lymphocyte Infusion

Complete or partial depletion of T cells from the graft has the ability to reduce the occurrence of GVHD [87]. However, crude T cell depletion strategies will also reduce the therapeutic GVT effect. By more specific T cell depletion strategies, such as the depletion of only CD161$^+$/Th/Tc17 cells, the GVT effect could be sustained by the non-GVHD–initiating T cells. Targeting the tissue homing properties, such as CCR6 or CXCR3, on these cells is a possibility, either by depletion of blockade of the homing capacity. However, this strategy will be less specific for Th/Tc17 depletion. In addition, depletion of the different DC subsets, such as pDCs and DNGR1$^+$ DCs, could also be a target, as specific depletion of DCs from the graft could inhibit the initiation of GVHD. However, to spare the GVT effect, depletion of specific DC subsets might be more favorable.

In Vivo Manipulation of Th/Tc17 Cell Differentiation

In addition to manipulation of the graft and donor lymphocyte infusion, in vivo targeting of Th/Tc17 cell populations could additionally lower the generation of these cells in time. As upregulation of RORγt is crucial in the generation of Th/Tc17, this could be used as a target to inhibit Th/Tc17 generation. This possibility has been shown in different mouse models, where less GVHD was observed upon adoptive transfer of RORγt$^{-/-}$ compared with WT T cells [24,25]. However, whether this is a direct effect of lacking Th/Tc17 cells, or whether this is due to the observed increase of Tregs should be investigated further A possible clinical application to apply this strategy in patients is a small molecule inhibitor for RORγt, as described by Huh et al. [88]. However, possible side effects should be taken into account, as studies of RORγt$^{-/-}$ mice have shown the role of RORγt in processes other than Th/Tc17 development, resulting in defects in lymphoid development. A putative safer option with similar effects could be induced by isoforms of retinoic acid, which inhibit Th17 formation and enhance Treg generation [17]. Together, targeting RORγt could be an option to prevent differentiation of Th/Tc17 cells, and thereby prevent the occurrence of GVHD.

PRR Inhibitors

Prevention of GVHD could also be achieved by preventing the activation of DCs by PAMPs and DAMPs. As these molecules have been implicated in GVHD pathobiology extensively, especially during the initiation phase, they could be promising cascade targets in the prevention of GVHD [38]. By inhibiting the PRRs, activation of pDCs and DNGR1$^+$ mDCs could be prevented, thereby suppressing the Th/Tc17 activation.

Cytokine Pathway Inhibition

An alternative approach to inhibit Th/Tc17 responses would be to influence cytokine cascades involved in the IL17 pathway. Blocking IL1β, which is a major player in GVHD, with canakinumab (IL1β antibody) or its receptor with anakinra (recombinant IL1R antagonist), could interfere with the activation of Th17 cells [89]. However, the blockade of IL1β has shown some contradictory results in GVHD treatment [90]. Blocking IL6 signaling could also contribute to preventing GVHD [91,92]. Nevertheless, blockade of IL6 has not resulted in clear effects on Th/Tc17 responses. A probable mechanism by which blockade of IL6 could contribute to the prevention of GVHD is the increased levels of Tregs observed, with the supplementary suppression of Th/Tc1 and Th/Tc17–mediated tissue damage [92,93]. On the other hand, promising results have been obtained by anti-IL17 (ixekizumab) and anti-IL17 receptor (brodalumab) in the treatment of the IL17-driven skin disease, psoriasis [94,95]. Although blocking IL17 using secukinumab (IL17-blocking antibody) was associated with increased infections in Crohn's disease patients, only local and no systemic fungal complications were observed, showing the feasibility to safely interfere with Th17 responses [96]. Promising results have also been found by depleting the p40 subunit of IL12 and IL23 with ustekinemab (IL12/IL23–blocking antibody), which is approved for the treatment of psoriasis. A recent case report showed the potency of ustekinemab in GVHD treatment, as a complete remission was observed after treatment of steroid-resistant GVHD with this blocking antibody [97]. However, this also coincided with a increase in Tregs, again suggesting that the effect is not solely Th/Tc17–lineage specific. Depletion of IL12 (p70) and IL23 could also be achieved using a small molecule inhibitor, which also for psoriasis, resulted in lower IL17 levels [98]. Moreover, molecules downstream of the cytokine signaling, such as STAT3, could also be considered possible targets. Combined, blockades of signaling pathways could be used for preventing and/or treating GVHD, blocking the Th/Tc17 initiation.

CONCLUSION AND FUTURE PERSPECTIVES

In recent years, new and exciting insights have been gained in the pathogenesis of GVHD, and increasingly, a role for IL17-producing cells and the Th/Tc17 pathway has been acknowledged. In the future, it might be proven that the specific context of allo-SCT determines whether IL17-producing T cells play an important role or not. As IL17-producing cells are mainly involved in epithelial barrier defenses of gut, lung, and skin, organs where GVHD occurs, it can be anticipated that the role of the Th/Tc17 pathway in GVHD is greatest in the presence of tissue damage (eg, resulting from myeloablative and reduced-intensity conditioning), microbial exposure (eg, infection or gut translocation), and the release of proinflammatory cytokines including IL1β, IL6, and IL23. Consequently, IL17-producing cells emerge and activate early on; that is, during or directly after the conditioning-induced tissue damage and innate immune activation. Thereafter, they set the stage for full-blown GVHD by inducing aggravated tissue inflammation, immune cell recruitment, and priming alloreactive Th1/Tc1 responses. Being an early event in GVHD, the Th/Tc17
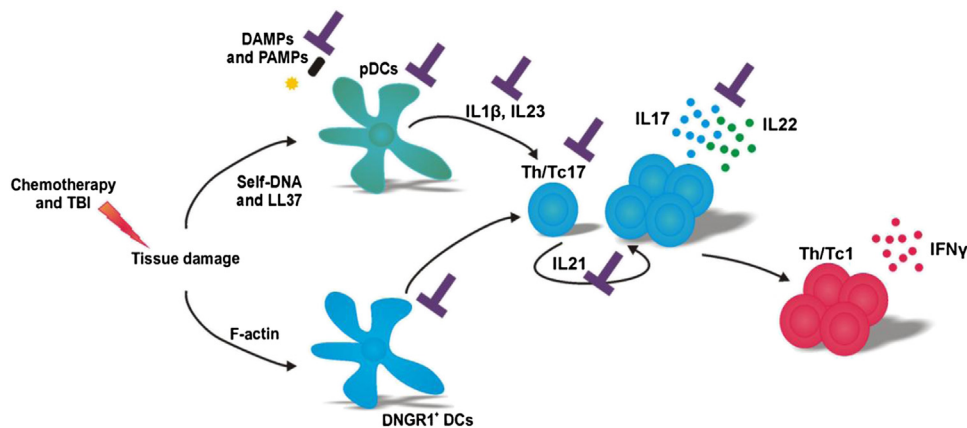
Figure 2. Th/Tc17 pathway involved in GVHD and its potential targets. TBI indicates total body irradiation; F-actin, filament actin; DNGR1, DC NK lectin group receptor; DC, dendritic cell. DAMP, danger-associated molecular patterns; PAMP, pathogen-associated molecular patterns, Th/Tc, helper T cell/cytotoxic T cell.

pathway seems to be both a logical and suitable target for preventive interventions, and this is most appealing as treatment of manifested acute GVHD remains difficult, with 50% being steroid refractory, and resulting in a high mortality.

Many factors are involved in the differentiation, expansion and activation of IL17-producing T cells in allo-SCT recipients. Different DC subsets, such as pDCs and DNGR1+ mDCs, have the capability to activate Th/Tc17 cells through the release of cytokines. Pivotal cytokines include IL1β, IL6, IL23, and TGFβ, which are known to drive the differentiation and expansion of IL17-producing T cells, and these cytokines have been shown to be elevated in patients after allo-SCT. Potent activators of these DC subtypes are motifs that are released on tissue damage (and microbial exposure) during allo-SCT. These motifs aggravate the Th/Tc17 response via activation of various PRRs, thereby initiating and perpetuating GVHD.

At all these different levels, interventions are possible (Figure 2). Attractive approaches are preventing the occurrence of tissue damage by using less toxic conditioning, and inhibiting Th/Tc17 generation by scavenging PAMPs and DAMPs that activate DCs, modulating the PRR pathways, and/or inhibiting key cytokines with monoclonal antibodies, eg, IL1β with anakinra and canakinumab and IL23 with ustekinumab.

In addition, Th/Tc17 can be targeted more specifically by depleting them from the graft, inhibiting their tissue-homing properties, or inhibiting their effector cytokines and related receptors, especially IL17, with ixekizumab and brodalumab, for example.

Although many new preventive strategies seem promising, further investigations are warranted. Still, many question remain to be answered on the role of different subsets of lymphocytes, including Th/Tc17, in acute GVHD. Nevertheless, the insights we now gain rapidly on the IL17-producing T cells open up new ways that can, and should be, explored for the prevention and treatment of GVHD. This way, we could enhance the outcome of allo-SCT and improve the quality-of-life of hematology patients.

REFERENCES

1. Shlomchik WD. Graft-versus-host disease. *Nat Rev Immunol.* 2007;7: 340-352.
2. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet.* 2009;373:1550-1561.
3. Hill GR, Teshima T, Gerbitz A, et al. Differential roles of IL-1 and TNF-alpha on graft-versus-host disease and graft versus leukemia. *J Clin Invest.* 1999;104:459-467.
4. Penack O, Holler E, van den Brink MR. Graft-versus-host disease: regulation by microbe-associated molecules and innate immune receptors. *Blood.* 2010;115:1865-1872.
5. Nikolic B, Lee S, Bronson RT, Grusby MJ, et al. Th1 and Th2 mediate acute graft-versus-host disease, each with distinct end-organ targets. *J Clin Invest.* 2000;105:1289-1298.
6. Bossard C, Malard F, Arbez J, et al. Plasmacytoid dendritic cells and Th17 immune response contribution in gastrointestinal acute graft-versus-host disease. *Leukemia.* 2012;26:1471-1474.
7. Carlson MJ, West ML, Coghill JM, et al. In vitro-differentiated TH17 cells mediate lethal acute graft-versus-host disease with severe cutaneous and pulmonary pathologic manifestations. *Blood.* 2009;113:1365-1374.
8. Dander E, Balduzzi A, Zappa G, et al. Interleukin-17-producing T-helper cells as new potential player mediating graft-versus-host disease in patients undergoing allogeneic stem-cell transplantation. *Transplantation.* 2009;88:1261-1272.
9. van der Waart AB, van der Velden WJ, van Halteren AG, et al. Decreased levels of circulating IL17-producing CD161+CCR6+ T cells are associated with graft-versus-host disease after allogeneic stem cell transplantation. *PLoS One.* 2012;7:e50896.
10. Koenen HJ, Smeets RL, Vink PM, et al. Human CD25highFoxp3pos regulatory T cells differentiate into IL-17-producing cells. *Blood.* 2008; 112:2340-2352.
11. Ivanov II, McKenzie BS, Zhou L, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell.* 2006;126:1121-1133.
12. Durant L, Watford WT, Ramos HL, et al. Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. *Immunity.* 2010;32:605-615.
13. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol.* 2007;8:942-949.
14. Wilson NJ, Boniface K, Chan JR, et al. Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol.* 2007;8:950-957.
15. Langrish CL, Chen Y, Blumenschein WM, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med.* 2005;201:233-240.
16. Zhou L, Ivanov II, Spolski R, et al. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol.* 2007;8:967-974.
17. Elias KM, Laurence A, Davidson TS, et al. Retinoic acid inhibits Th17 polarization and enhances FoxP3 expression through a Stat-3/Stat-5 independent signaling pathway. *Blood.* 2008;111:1013-1020.
18. Veldhoen M, Hirota K, Westendorf AM, et al. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature.* 2008;453:106-109.
19. Weaver CT, Elson CO, Fouser LA, Kolls JK. The Th17 pathway and inflammatory diseases of the intestines, lungs, and skin. *Annu Rev Pathol.* 2013;8:477-512.

20. Ersvaer E, Melve GK, Bruserud O. Future perspectives: should Th17 cells be considered as a possible therapeutic target in acute myeloid leukemia patients receiving allogeneic stem cell transplantation? *Cancer Immunol Immunother.* 2011;60:1669-1681.
21. Kappel LW, Goldberg GL, King CG, et al. IL-17 contributes to CD4-mediated graft-versus-host disease. *Blood.* 2009;113:945-952.
22. Yi T, Zhao D, Lin CL, et al. Absence of donor Th17 leads to augmented Th1 differentiation and exacerbated acute graft-versus-host disease. *Blood.* 2008;112:2101-2110.
23. Yi T, Chen Y, Wang L, et al. Reciprocal differentiation and tissue-specific pathogenesis of Th1, Th2, and Th17 cells in graft-versus-host disease. *Blood.* 2009;114:3101-3112.
24. Yu Y, Wang D, Liu C, et al. Prevention of GVHD while sparing GVL effect by targeting Th1 and Th17 transcription factor T-bet and RORgammat in mice. *Blood.* 2011;118:5011-5020.
25. Fulton LM, Carlson MJ, Coghill JM, et al. Attenuation of acute graft-versus-host disease in the absence of the transcription factor ROR-gammat. *J Immunol.* 2012;189:1765-1772.
26. Billerbeck E, Kang YH, Walker L, et al. Analysis of CD161 expression on human CD8+ T cells defines a distinct functional subset with tissue-homing properties. *Proc Natl Acad Sci U S A.* 2010;107:3006-3011.
27. Hanash AM, Dudakov JA, Hua G, et al. Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. *Immunity.* 2012;37:339-350.
28. Caproni M, Antiga E, Melani L, et al. Serum levels of IL-17 and IL-22 are reduced by etanercept, but not by acitretin, in patients with psoriasis: a randomized-controlled trial. *J Clin Immunol.* 2009;29:210-214.
29. Geboes L, Dumoutier L, Kelchtermans H, et al. Proinflammatory role of the Th17 cytokine interleukin-22 in collagen-induced arthritis in C57BL/6 mice. *Arthritis Rheum.* 2009;60:390-395.
30. Couturier M, Lamarthee B, Arbez J, et al. IL-22 deficiency in donor T cells attenuates murine acute graft-versus-host disease mortality while sparing the graft-versus-leukemia effect. *Leukemia.* 2013;27: 1527-1537.
31. Liu Y, Cai Y, Dai L, et al. The expression of Th17-associated cytokines in human acute graft-versus-host disease. *Biol Blood Marrow Transplant.* 2013;19:1421-1429.
32. Elmaagacli AH, Koldehoff M, Landt O, Beelen DW. Relation of an interleukin-23 receptor gene polymorphism to graft-versus-host disease after hematopoietic-cell transplantation. *Bone Marrow Transplant.* 2008;41:821-826.
33. Gruhn B, Intek J, Pfaffendorf N, et al. Polymorphism of interleukin-23 receptor gene but not of NOD2/CARD15 is associated with graft-versus-host disease after hematopoietic stem cell transplantation in children. *Biol Blood Marrow Transplant.* 2009;15:1571-1577.
34. Broady R, Yu J, Chow V, et al. Cutaneous GVHD is associated with the expansion of tissue-localized Th1 and not Th17 cells. *Blood.* 2010;116: 5748-5751.
35. Nistala K, Adams S, Cambrook H, et al. Th17 plasticity in human autoimmune arthritis is driven by the inflammatory environment. *Proc Natl Acad Sci U S A.* 2010;107:14751-14756.
36. Maggi L, Santarlasci V, Capone M, et al. CD161 is a marker of all human IL-17-producing T-cell subsets and is induced by RORC. *Eur J Immunol.* 2010;40:2174-2181.
37. Broen K, van der Waart AB, Greupink-Draaisma A, et al. Polymorphisms in CCR6 are associated with chronic graft-versus-host disease and invasive fungal disease in matched-related hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* 2011;17: 1443-1449.
38. Schutyser E, Struyf S, Van DJ. The CC chemokine CCL20 and its receptor CCR6. *Cytokine Growth Factor Rev.* 2003;14:409-426.
39. Hirata T, Osuga Y, Takamura M, et al. Recruitment of CCR6-expressing Th17 cells by CCL 20 secreted from IL-1 beta-, TNF-alpha-, and IL-17A-stimulated endometriotic stromal cells. *Endocrinology.* 2010;151: 5468-5476.
40. Schmuth M, Neyer S, Rainer C, et al. Expression of the C-C chemokine MIP-3 alpha/CCL20 in human epidermis with impaired permeability barrier function. *Exp Dermatol.* 2002;11:135-142.
41. Piper KP, Horlock C, Curnow SJ, et al. CXCL10-CXCR3 interactions play an important role in the pathogenesis of acute graft-versus-host disease in the skin following allogeneic stem-cell transplantation. *Blood.* 2007;110:3827-3832.
42. Croudace JE, Inman CF, Abbotts BE, et al. Chemokine-mediated tissue recruitment of CXCR3+ CD4+ T cells plays a major role in the pathogenesis of chronic GVHD. *Blood.* 2012;120:4246-4255.
43. Olsen T, Rismo R, Cui G, et al. TH1 and TH17 interactions in untreated inflamed mucosa of inflammatory bowel disease, and their potential to mediate the inflammation. *Cytokine.* 2011;56:633-640.
44. Shen W, Durum SK. Synergy of IL-23 and Th17 cytokines: new light on inflammatory bowel disease. *Neurochem Res.* 2010;35:940-946.
45. Ratajczak P, Janin A, Peffault de LR, et al. Th17/Treg ratio in human graft-versus-host disease. *Blood.* 2010;116:1165-1171.
46. Shlomchik WD, Couzens MS, Tang CB, et al. Prevention of graft versus host disease by inactivation of host antigen-presenting cells. *Science.* 1999;285:412-415.
47. Stenger EO, Turnquist HR, Mapara MY, Thomson AW. Dendritic cells and regulation of graft-versus-host disease and graft-versus-leukemia activity. *Blood.* 2012;119:5088-5103.
48. Vakkila J, Thomson AW, Hovi L, et al. Circulating dendritic cell subset levels after allogeneic stem cell transplantation in children correlate with time post transplant and severity of acute graft-versus-host disease. *Bone Marrow Transplant.* 2005;35:501-507.
49. Isaksson M, Ardesjo B, Ronnblom L, et al. Plasmacytoid DC promote priming of autoimmune Th17 cells and EAE. *Eur J Immunol.* 2009;39: 2925-2935.
50. Yu CF, Peng WM, Oldenburg J, et al. Human plasmacytoid dendritic cells support Th17 cell effector function in response to TLR7 ligation. *J Immunol.* 2010;184:1159-1167.
51. Turtle CJ, Delrow J, Joslyn RC, et al. Innate signals overcome acquired TCR signaling pathway regulation and govern the fate of human CD161hi CD8{alpha}+ semi-invariant T cells. *Blood.* 2011;118: 2752-2762.
52. Sisirak V, Vey N, Vanbervliet B, et al. CCR6/CCR10-mediated plasmacytoid dendritic cell recruitment to inflamed epithelia after instruction in lymphoid tissues. *Blood.* 2011;118:5130-5140.
53. Lande R, Gregorio J, Facchinetti V, et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature.* 2007;449: 564-569.
54. Gilliet M, Lande R. Antimicrobial peptides and self-DNA in autoimmune skin inflammation. *Curr Opin Immunol.* 2008;20:401-407.
55. Frohm M, Agerberth B, Ahangari G, et al. The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *J Biol Chem.* 1997;272: 15258-15263.
56. Joffre OP, Sancho D, Zelenay S, et al. Efficient and versatile manipulation of the peripheral CD4+ T-cell compartment by antigen targeting to DNGR-1/CLEC9A. *Eur J Immunol.* 2010;40:1255-1265.
57. Poulin LF, Reyal Y, Uronen-Hansson H, et al. DNGR-1 is a specific and universal marker of mouse and human Batf3-dependent dendritic cells in lymphoid and nonlymphoid tissues. *Blood.* 2012;119:6052-6062.
58. Ahrens S, Zelenay S, Sancho D, et al. F-actin is an evolutionarily conserved damage-associated molecular pattern recognized by DNGR-1, a receptor for dead cells. *Immunity.* 2012;36:635-645.
59. Murphy S, Nguyen VH. Role of gut microbiota in graft-versus-host disease. *Leuk Lymphoma.* 2011;52:1844-1856.
60. Zeiser R, Penack O, Holler E, Idzko M. Danger signals activating innate immunity in graft-versus-host disease. *J Mol Med (Berl).* 2011;89: 833-845.
61. Jones JM, Wilson R, Bealmear PM. Mortality and gross pathology of secondary disease in germfree mouse radiation chimeras. *Radiat Res.* 1971;45:577-588.
62. van Bekkum DW, Roodenburg J, Heidt PJ, van der Waaij D. Mitigation of secondary disease of allogeneic mouse radiation chimeras by modification of the intestinal microflora. *J Natl Cancer Inst.* 1974;52:401-404.
63. Jenq RR, Ubeda C, Taur Y, et al. Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. *J Exp Med.* 2012;209:903-911.
64. Akpek G, Joseph R, Gunay C, et al. Frequent detection of herpes simplex virus antigen in skin and peripheral blood CD34+ mononuclear cells from patients with graft-versus-host disease. *Biol Blood Marrow Transplant.* 2013;19:529-537.
65. Cantoni N, Hirsch HH, Khanna N, et al. Evidence for a bidirectional relationship between cytomegalovirus replication and acute graft-versus-host disease. *Biol Blood Marrow Transplant.* 2010;16:1309-1314.
66. Beelen DW, Elmaagacli A, Muller KD, et al. Influence of intestinal bacterial decontamination using metronidazole and ciprofloxacin or ciprofloxacin alone on the development of acute graft-versus-host disease after marrow transplantation in patients with hematologic malignancies: final results and long-term follow-up of an open-label prospective randomized trial. *Blood.* 1999;93:3267-3275.
67. van der Velden WJ, Netea MG, de Haan AF, et al. Role of the mycobiome in human acute graft-versus-host disease. *Biol Blood Marrow Transplant.* 2013;19:329-332.
68. Marr KA, Seidel K, Slavin MA, et al. Prolonged fluconazole prophylaxis is associated with persistent protection against candidiasis-related death in allogeneic marrow transplant recipients: long-term follow-up of a randomized, placebo-controlled trial. *Blood.* 2000;96: 2055-2061.
69. Brandon JA, Jennings CD, Kaplan AM, Bryson JS. Development of a T(H)17 immune response during the induction of murine syngeneic graft-versus-host disease. *Cytokine.* 2010;52:265-273.
70. Kullberg MC, Andersen JF, Gorelick PL, et al. Induction of colitis by a CD4+ T cell clone specific for a bacterial epitope. *Proc Natl Acad Sci U S A.* 2003;100:15830-15835.
71. Jankovic D, Ganesan J, Bscheider M, et al. The Nlrp3 inflammasome regulates acute graft-versus-host disease. *J Exp Med.* 2013;210: 1899-1910.
72. Dusseaux M, Martin E, Serriari N, et al. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. *Blood.* 2011;117:1250-1259.

73. Smeekens SP, van de Veerdonk FL, van der Meer JW, et al. The Candida Th17 response is dependent on mannan-and beta-glucan-induced prostaglandin E2. *Int Immunol.* 2010;22:889-895.

74. Jarrossay D, Napolitani G, Colonna M, et al. Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. *Eur J Immunol.* 2001;31:3388-3393.

75. Bauer S, Kirschning CJ, Hacker H, et al. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc Natl Acad Sci U S A.* 2001;98:9237-9242.

76. Parcina M, Wendt C, Goetz F, et al. Staphylococcus aureus-induced plasmacytoid dendritic cell activation is based on an IgG-mediated memory response. *J Immunol.* 2008;181:3823-3833.

77. Min CK, Lee WY, Min DJ, et al. The kinetics of circulating cytokines including IL-6, TNF-alpha, IL-8 and IL-10 following allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2001;28:935-940.

78. Visentainer JE, Lieber SR, Persoli LB, et al. Serum cytokine levels and acute graft-versus-host disease after HLA-identical hematopoietic stem cell transplantation. *Exp Hematol.* 2003;31:1044-1050.

79. Liem LM, van Houwelingen HC, Goulmy E. Serum cytokine levels after HLA-identical bone marrow transplantation. *Transplantation.* 1998;66:863-871.

80. Sakata N, Yasui M, Okamura T, et al. Kinetics of plasma cytokines after hematopoietic stem cell transplantation from unrelated donors: the ratio of plasma IL-10/sTNFR level as a potential prognostic marker in severe acute graft-versus-host disease. *Bone Marrow Transplant.* 2001;27:1153-1161.

81. Cho BS, Lim JY, Yahng SA, et al. Circulating IL-17 levels during the peri-transplant period as a predictor for early leukemia relapse after myeloablative allogeneic stem cell transplantation. *Ann Hematol.* 2012;91:439-448.

82. Espinoza JL, Takami A, Nakata K, et al. A genetic variant in the IL-17 promoter is functionally associated with acute graft-versus-host disease after unrelated bone marrow transplantation. *PLoS One.* 2011;6:e26229.

83. Hanash AM, Kappel LW, Yim NL, et al. Abrogation of donor T-cell IL-21 signaling leads to tissue-specific modulation of immunity and separation of GVHD from GVL. *Blood.* 2011;118:446-455.

84. Hippen KL, Bucher C, Schirm DK, et al. Blocking IL-21 signaling ameliorates xenogeneic GVHD induced by human lymphocytes. *Blood.* 2012;119:619-628.

85. Bucher C, Koch L, Vogtenhuber C, et al. IL-21 blockade reduces graft-versus-host disease mortality by supporting inducible T regulatory cell generation. *Blood.* 2009;114:5375-5384.

86. Deeg HJ. How I treat refractory acute GVHD. *Blood.* 2007;109:4119-4126.

87. Bayraktar UD, de Lima M, Saliba RM, et al. Ex vivo T cell-depleted versus unmodified allografts in patients with acute myeloid leukemia in first complete remission. *Biol Blood Marrow Transplant.* 2013;19:898-903.

88. Huh JR, Leung MW, Huang P, et al. Digoxin and its derivatives suppress TH17 cell differentiation by antagonizing RORgammat activity. *Nature.* 2011;472:486-490.

89. Church LD, McDermott MF. Canakinumab, a fully-human mAb against IL-1beta for the potential treatment of inflammatory disorders. *Curr Opin Mol Ther.* 2009;11:81-89.

90. Antin JH, Weisdorf D, Neuberg D, et al. Interleukin-1 blockade does not prevent acute graft-versus-host disease: results of a randomized, double-blind, placebo-controlled trial of interleukin-1 receptor antagonist in allogeneic bone marrow transplantation. *Blood.* 2002;100:3479-3482.

91. Chen X, Das R, Komorowski R, et al. Blockade of interleukin-6 signaling augments regulatory T-cell reconstitution and attenuates the severity of graft-versus-host disease. *Blood.* 2009;114:891-900.

92. Tawara I, Koyama M, Liu C, et al. Interleukin-6 modulates graft-versus-host responses after experimental allogeneic bone marrow transplantation. *Clin Cancer Res.* 2011;17:77-88.

93. Pesce B, Soto L, Sabugo F, et al. Effect of interleukin-6 receptor blockade on the balance between regulatory T cells and T helper type 17 cells in rheumatoid arthritis patients. *Clin Exp Immunol.* 2013;171:237-242.

94. Leonardi C, Matheson R, Zachariae C, et al. Anti-interleukin-17 monoclonal antibody ixekizumab in chronic plaque psoriasis. *N Engl J Med.* 2012;366:1190-1199.

95. Papp KA, Leonardi C, Menter A, et al. Brodalumab, an anti-interleukin-17-receptor antibody for psoriasis. *N Engl J Med.* 2012;366:1181-1189.

96. Hueber W, Sands BE, Lewitzky S, et al. Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. *Gut.* 2012;61:1693-1700.

97. Pidala J, Perez L, Beato F, Anasetti C. Ustekinumab demonstrates activity in glucocorticoid-refractory acute GVHD. *Bone Marrow Transplant.* 2012;47:747-748.

98. Wada Y, Cardinale I, Khatcherian A, et al. Apilimod inhibits the production of IL-12 and IL-23 and reduces dendritic cell infiltration in psoriasis. *PLoS One.* 2012;7:e35069.