Translational opportunities for targeting the Th17 axis in acute graft-vs.-host disease

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Allogeneic stem cell transplantation (allo-SCT) is a curative therapy for different life-threatening malignant and non-malignant hematologic disorders. Acute graft-vs.-host disease (aGVHD) and particularly gastrointestinal aGVHD remains a major source of morbidity and mortality following allo-SCT, which limits the use of this treatment in a broader spectrum of patients. Better understanding of aGVHD pathophysiology is indispensable to identify new therapeutic targets for aGVHD prevention and therapy. Growing amount of data suggest a role for T helper (Th)17 cells in aGVHD pathophysiology. In this review, we will discuss the current knowledge in this area in animal models and in humans. We will then describe new potential treatments for aGVHD along the Th17 axis.

INTRODUCTION

Allogeneic stem cell transplantation (allo-SCT) is a curative therapy for different life-threatening hematologic malignancies. The therapeutic efficacy of allo-SCT relies on the combination of the cytoreductive effect of the conditioning chemotherapy and/or radiotherapy and of the graft-vs.-tumor effect mediated by the donor's immunocompetent cells (CD8+ and CD4+ T cells, natural killer cells and dendritic cells). However, the beneficial effect of graft-vs.-tumor effect is counterbalanced by the immunological recognition and destruction of host tissues by the donor's immune effectors, termed graft-vs.-host disease (GVHD). GVHD remains a major source of morbidity and mortality following allo-SCT. Gooley *et al.*[1] recently reported a substantial reduction in death related to allo-SCT and an increased long-term survival. Similarly, we reported[2] a significant reduction of non-relapse mortality significantly and improvement of overall survival in the 2001–2010 period, compared with 1983–2000 period, while the incidence of acute GVHD (aGVHD) remained stable, and the incidence of extensive chronic GVHD increased, during the same period.[2] Therefore, it is essential to improve GVHD management.

Consequently, there have been several attempts to develop biological biomarkers to predict GVHD onset or responsiveness to treatment.[3,4] This would allow a more stringent monitoring and intensified prophylaxis or curative treatment of GVHD in those patients. Furthermore, recent progress in medical imaging test and endoscopic techniques, such as contrast-enhanced ultrasound or probe-based confocal endomicroscopy, may allow an earlier and more specific diagnosis of GVHD,[5,6] particularly for gastrointestinal aGVHD (reviewed in Malard and Mohty[7]). Finally, identification of new therapeutic targets and development of new immunosuppressive therapy are indispensables to further improve GVHD management.

The pathophysiology of aGVHD is a multistep process.[8,9] In the first step, the conditioning regimen (chemotherapy and/or total body irradiation) leads to host tissue damage, release of proinflammatory cytokines, and increased expression of major and minor histocompatibility antigens and co-stimulatory molecules on host antigen-presenting cells. In the second step, donor-derived CD4+ and CD8+ T cells are activated by host antigen-presenting cell and migrate into GVHD target tissues (gastrointestinal tract, skin, and liver). In the third step, cellular mediators (such as cytotoxic T lymphocytes, activated macrophages, and natural killer cells) and inflammatory cytokines act synergistically to enhance

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Received 24 June 2015; accepted 2 December 2015; published online 27 January 2016. doi:10.1038/mi.2015.143

target tissue destruction.[8,9] For a long time, we considered that a particular subset of CD4+ T helper (Th) cells, Th1 cells, was at play during the effector phase of aGVHD.[10,11] However, the identification of a new Th subset, Th17 cells, raised the question of their role in aGVHD. Therefore, in this review we will discuss the most recent data on the contribution of these Th17 cells and Th17-related cytokines in aGVHD pathophysiology.

Th17 CELLS

Th17 cells differentiation

In 2005, two seminal studies[12,13] showed in a mouse model that the development of Th17 cells from naive precursors was independent of Th1- and Th2-specific transcription factors (Tbet and Gata-3), leading to the establishment of the Th17 lineage as independent and distinct from the Th1 and Th2 lineages.[12,13] Another group reported that mouse Th17 cells uniquely expressed a transcription factor termed retinoid acid-related orphan receptor (ROR)γt (encoded by the gene *Rorc*).[14]

Besides RORγt, STAT3 is the second transcription factor required for Th17 cells differentiation. STAT3 has pleiotropic functions as a transcriptional activator for *Rorc*, *IL-17*, *IL-17F*, *IL-23R*, and others genes implied in Th17 cell differentiation or survival in murine models.[15] Several cytokines have a role in Th17 cell differentiation, upon the control of the RORγt and STAT3 transcription factors.

In murine models, interleukin (IL)-6 has an essential role in this process by activating STAT3,[16] which directly drives the transcription of Th17 lineage specific genes[15] and suppresses transforming growth factor (TGF)β-induced forkhead box P3 (FOXP3) expression, thereby inhibiting regulatory T-cell (Treg) development.[17] IL-6 also induces the expression of IL-1R1 by mouse Th17 cells.[18] IL-1β, through its receptor IL-1R1, promotes the transcription factor interferon-regulatory factor 4, which reinforces the expression of RORγt, and enhances Th17 proliferation in experimental models.[18] Therefore, these data suggest that if IL-6 directly drives the differentiation of Th17 cells, IL-1β enhances the expansion of these cells. Alternatively, IL-21 selectively induces the phosphorylation of STAT3 and replacement of IL-6 with IL-21 in combination with TGFβ, interferon γ (IFNγ), and IL-4, was very effective to induce high level of IL-17-producing cells in a mouse model.[19] Furthermore, IL-21 is also produced by Th17 cells, promoting self-maintenance of Th17 cells.[20] Regarding TGFβ, although the data suggest that TGFβ is required for Th17 cells differentiation in mouse models,[21,22] it probably does not act as a direct Th17 cell-inducing factor, but rather allows Th17 cells differentiation indirectly by suppressing alternative cell fates.[23,24]

In humans, although the role of IL-6 and IL-1β is well established on *in vitro* cultures of human T cells,[25–27] the contribution of TGFβ to Th17 cells differentiation remains a matter of debate. Although some groups have shown that TGFβ is necessary for *in vitro* Th17 cell differentiation,[28,29] others found that Th17 cells could differentiate without TGFβ, upon stimulation with a cocktail of IL-6, IL-1β, and IL-23.[25–27] However, these are *in vitro* data, and it is difficult to draw definitive conclusion regarding the role of TGFβ for Th17 cells differentiation under *in vivo* inflammatory conditions in human. Besides, IL-21, produced by a number of T cells and the NK cell subset, is also involved in human Th17 cells differentiation via STAT3 signaling.[29]

IL-23 is another key cytokine for Th17 cells commitment. Therefore, despite the combination of IL-6 and TGFβ being sufficient to drive Th17 cells differentiation, the cells generate by this combination fail to induce pathogenicity in mouse models.[30] However, IL-6 and TGFβ induce IL-23R expression,[16] and subsequent exposure to IL-23 stabilizes the phenotype[31] and expands the pathogenicity of Th17 cells.[27,30,32]

Th17 cell plasticity

Despite initial thoughts that CD4+ naive T cells differentiate into terminal phenotypes in a rigid process, it is now quite well-accepted that, depending on the cytokine milieu, certain Th subset can adopt a mixed phenotype or switch entirely to the transcription and cytokine profile of another lineage. Given that TGFβ suppresses Th1 and Th2 differentiation in mouse,[23,24] both Th17 and induced Treg (iTreg) development is favored in its presence. Therefore, after T cell receptor (TCR) engagement, CD4+ T cell differentiation into Th17 or iTreg in the presence of TGFβ will depend on the cytokine environment: in the absence of proinflammatory signals, naive T cells will differentiate into iTregs, whereas IL-6 will promote Th17 cell development both in mouse models and *in vivo* in human.[23,24,33] The presence of all-*trans* retinoid acid in the microenvironment seems to inhibit Th17 and promote iTreg development in mouse, in part, at least, by antagonizing the effect of IL-6.[34-37] Therefore, Yang *et al.*[38] demonstrated in a mouse model that upon IL-6 stimulation, both natural Treg and iTreg repress Foxp3 and produce IL-17, suggesting that fully differentiated Treg could be converted into Th17-like cells. In human, Koenen *et al.* have shown *in vivo* that circulating Foxp3+ Treg can differentiate into IL-17-producing cells RORγt+, given that antigen-presenting cell, in particular monocyte, and the cytokine IL-2 or IL-15 are present.[39] Of note, this differentiation process was enhanced by exogenous IL-1β, IL-23, and IL-21, whereas IL-6 or TGFβ did not affect the emergence of IL-17-producing cells.[39] The *in vivo* existence of hybrid Treg/Th17 cells in human has been established in inflamed intestinal mucosa of patients with Crohn disease.[40] These cells express Foxp3 and RORγt and produce IL-17, however, unlike conventional Th17 cells, they functionally retain their suppressive activity *in vitro*.[40] A similar TCRβ chain variable region between Treg/Th17 and Treg cells suggest that those Treg/Th17 cells arise from Treg cell when exposed to the inflammatory signals present in inflamed Crohn disease tissue.[40] Similarly, Voo *et al.*[41] reported that human peripheral blood and lymphoid tissue contain a subpopulation of Foxp3+ Treg cells that coexpress RORγt and

have the capacity to produce IL-17 upon activation. In contrast, the conversion of Th17 cells into Treg has not been reported so far.

Similarly, the Th1 and the Th17 pathways share a common point: a critical event in the late development of both pathways is the induction of a receptor for an IL-12 cytokine family member: IL-12 for Th1 and IL-23 for Th17. These receptors share a common subunit, the IL-12Rβ1, associated with the IL-12Rβ2 to form the IL-12 receptor,[42] and with the IL-23R to form the IL-23 receptor. Similarly to the IL-23R upregulation during Th17 cell differentiation,[16] IL-12Rβ2 is upregulated during Th1 cell differentiation.[43] However, during their differentiation, Th17 cells also weakly express the IL-12Rβ1. Therefore, both *in vivo* mouse data and *in vitro* human studies have shown that depending on the balance between the cytokines present in the milieu, IL-12 can induce the conversion of Th17 cells into IFNγ-producing Th1-like cells.[44,45] These cells maintain their IL-17 memory upon subsequent culture.[44,45] Finally, *in vivo* existence in Crohn disease patients of a Th1/Th17 hybrid subset that arises from the modulation of Th17 cells by IL-12 has been established.[45]

Regarding Th2 subset, *in vitro* culture of mouse T cells under mixed Th1 and Th2 conditions resulted in a continuum of mixed phenotypes with subpopulations of cells expressing only IFNγ, only IL-4 or both cytokines, correlating with the expression level of Tbet and Gata-3.[46] The *in vivo* existence of Th1/Th2 hybrid subset has been confirmed in a mouse model; during infection with *Heligmosomoides polygyrus*, a parasite that triggers a strong Th2 response, Th1/Th2 hybrid cells, that express simultaneously Tbet and Gata-3, have been observed.[47] Hegazy *et al.* demonstrated in a murine model that injection of Th1 cell-promoting lymphocytic choriomeningitis virus reprogrammed otherwise stably committed Gata-3+ Th2 cells to adopt a Gata-3+ Tbet+ and IL-4+ IFNγ+ "Th1/Th2" phenotype that was maintained *in vivo* for months.[48] Moreover, Th2 cell reprogramming into hybrid Th1/Th2 subset required TCR stimulation and concerted type 1 and 2 IFN and IL-12 signals.[48] Finally, since the presence of IL-4, during *in vitro* naive T-cell activation, inhibits RORγt expression and IL-17 production, hybrid Th2/Th17 were thought not to exist. However, Califano *et al.*[49] have recently shown in a mouse model of autoimmune encephalomyelitis that Th17 deficient in the transcription factor BCL11B upregulate the Th2-associated proteins Gata-3 and IL-4 without decreasing RORγt and IL-17 level. So far, no data has been reported on the existence of Th1/Th2 or Th2/Th17 hybrid subset in human.

Th17 role

Th17 cells produce several cytokines, which are not typically produced by Th1 and Th2 cells. These cytokines include IL-17A, IL-17F, IL-17A/F, IL-21, IL-22, granulocyte macrophage colony-stimulating factor or human IL-26, and many other factors.[50] Th17 are usually present in the lamina propria of the small intestine[14] and can be rapidly induced in other mucosal sites during infection.[51–53] Therefore, Th17 cells and Th17-related cytokine contribute to the host defense against a wide variety of pathogens, predominantly extracellular bacteria and fungal pathogens, in the epithelial barrier of gut, skin and lung.[50,54] Thus, once released, IL-17 and IL-22 act synergistically to enhance mucosal site defences by the production of antimicrobial peptide such as βdefensine-2 or S100 proteins.[55]

Finally, Th17 responses also contribute to the pathogenesis of some diseases.[50] Therefore, their contribution to the pathophysiology of several autoimmune and autoinflammatory diseases affecting epithelial barrier, such as psoriasis or inflammatory bowel disease, is well established.[50] Given that aGVHD involves mostly the gastrointestinal tract, the skin, and the liver, which contain epithelial barrier, Th17 contribution has been explored in aGVHD pathophysiology.

Th17 CELLS IN aGVHD

Studies in mouse models of aGVHD

The contribution of Th17 cells in aGVHD pathophysiology has been demonstrated in several mouse models. Lu *et al.* found that phosphorylation of STAT3, a transcriptional factor involved in Th17 cell differentiation,[15] was important during T cells alloactivation during aGVHD and that interference with STAT3 phosphorylation can inhibit T-cell activation and proliferation *in vitro* and aGVHD *in vivo*, suggesting a role for Th17 cells in aGVHD.[56] Thereafter, Carlson *et al.* and Iclozan *et al.* have shown that adoptive transfer of *in vitro* differentiated Th17 cells mediate IL-17-dependent lethal aGVHD with severe tissue lesions.[57,58] In a mouse model of aGVHD using IL-17 −/− donor CD4+ T cells, Kappel *et al.*[59] found that aGVHD development was significantly delayed compared with recipients of wild-type CD4+ T cells, although the overall GVHD mortality remained unaffected. They concluded that despite Il-17 being dispensable for aGVHD, it contributes to its early development.[59] In contrast, Yi *et al.*[60] reported on a similar model that transplantation of IL-17 −/− donor CD4+ T cells induced exacerbated aGVHD compared with wild-type CD4+ T cells, while administration of recombinant IL-17 and neutralizing IFNγ to the recipients given IL-17 −/− donor cells ameliorated aGVHD. Their conclusion was that donor Th17 cells ameliorate aGVHD through downregulation of Th1 cell differentiation.[60] Nevertheless, given the plasticity between Th17 and Th1 cells,[44,45] this result could be explained by an enhanced differentiation of Th1 cells in recipients given IL-17 −/− donor cells, and does not contradict a pathological role for Th17 cells in aGVHD. Therefore, Gartlan *et al.* recently identified a population of inflammatory CD8+ cytotoxic T (Tc) 17 cells (iTc17) that develops rapidly after allo-SCT and contribute to GVHD but failed to maintain lineage fidelity.[61]

Yi *et al.*[62] also showed that administration of donor CD4+ T cells depleted for both IFNγ and IL-4 (a Th2-related cytokine) resulted in augmented Th17 differentiation, and preferential, though not exclusive, aGVHD damage to the skin. Fulton *et al.*[63] have shown, in a major mismatch murine model, that

deletion of *Rorc* in both CD4+ and CD8+ donor T cells attenuated aGVHD and decreased tissue pathology in the colon, liver, and lung. Hill *et al.* have shown that use of granulocyte colony-stimulating factor for stem cell mobilization invoke Th17 responses rather than Th1/Th2 differentiation.[64] Therefore, while transplantation of granulocyte colony-stimulating factor-mobilized graft from wild-type or IL-17A−/− B6 donors resulted in identical GVHD outcome in models of aGVHD, transplantation of graft from IL-17A−/− BALB/c donors resulted in attenuated GVHD, suggesting a role for IL-17A in GVHD. However, in both recipients of B6 and BALB/c donor grafts, IL-17A promoted cutaneous GVHD with increased levels of both inflammation and fibrosis in the skin of wild-type grafts, suggesting that use of granulocyte colony-stimulating factor-mobilized grafts promoted sclerodermatous chronic GVHD. In a more relevant haploidentical murine transplantation model, *Rorc*−/− CD4+ T cells alone diminished the severity and the lethality of aGVHD.[63] Finally, Uryu *et al.*[65] recently reported that α-Mannan, a major component of fungal cell wall, induced donor T-cell polarization toward Th17, leading to exacerbated Th17pulmonary aGVHD in mice.

Some studies have also explored the role of cytokines implicated in Th17 cell differentiation. Therefore, inhibition of the IL-6 signaling pathway that drives Th17 cell differentiation by a way of antibody-mediated blockade of the IL-6 receptor markedly reduces pathologic damage attributable to GVHD.[66] This effect is accompanied by a significant reduction of Th1 and Th17 cells infiltrating aGVHD target tissues and a significant increase of Treg.[66] Similarly, Tawara *et al.*[67] reported that transplantation of *IL-6*−/− donor T cells or total inhibition of IL-6 with anti-IL-6 receptor monoclonal antibody lead to a marked decrease in aGVHD severity and prolonged survival. However, they failed to demonstrate a role of donor T cells in this effect.[67] Other authors focused on IL-23, a cytokine that stabilizes Th17 cell phenotype[31] and expands their pathogenicity.[27,30,32] Das *et al.*[68] have shown that donor antigen-presenting cells-derived IL-23 drive gastrointestinal aGVHD. The proinflammatory effect of IL-23 was reported to be dependent upon donor-derived secretion of IFNγ and not IL-17, despite IL-17 being significantly decreased in *IL-23*−/− compared with wild-type donors.[68] Furthermore, they have shown that under IL-23 blockade, the graft-vs.-leukemia effect was preserved.[69] Thompson *et al.*[70] have confirmed that absence of IL-23 in donor grafts reduced the severity of aGVHD and was associated with a decrease of IL-17. Th17 cells produce IL-21, involved in their differentiation,[20,29] promoting, therefore, their self-maintenance.[20] Transplantation with *IL-21R*−/− donor T cells resulted in less severe aGVHD, while sparing the graft-vs.-leukemia effect.[71–74] Furthermore, IL-21 blockade using a monoclonal antibody also decreased aGVHD.[75] In these studies, the protective effect of IL-21 signaling pathway blockade on aGVHD was associated with an expansion of Tregs, and no effect was observed on the IL-17 axis.[74,75] In a xenogeneic GVHD model, IL-21 blockade also significantly reduced aGVHD;[76] nevertheless, this reduction was associated with an increase in Tregs and a decrease of IL-17-producing cells.[76]

Several studies have explored the contribution of another Th17-related cytokine, IL-22, in aGVHD pathophysiology. IL-22 is structurally related to the IL-10 family, secreted by Th17 cells, but also by others αβT cells (Th1, Th22, and CD8+ αβT cells), γδT cells, natural killer T cells, and innate lymphoid cells.[77] IL-22 has been reported to exert both protective and inflammatory functions, most likely depending on the cytokine microenvironment and the tissue and/or the cell type involved.[78] Thus, while IL-22 has been shown to be protective in inflammatory bowel disease,[79] it is pathogenic in psoriasis[80] and rheumatoid arthritis.[81] In aGVHD, we have recently reported that IL-22 deficiency in donor T cells can decrease the severity of aGVHD while sparing the graft-vs.-leukemia effect.[82] Furthermore, weekly administration of IL-22, starting on day 0, aggravates aGVHD in animal models.[83] In contrast, Hanash *et al.*[84] showed that IL-22 produced by recipient innate lymphoid cell decreased aGVHD tissue damage by protecting intestinal stem cells. Therefore, according to the cell source (donor or patient), IL-22 may have an either protective or inflammatory effect in aGVHD. The IL-22 axis remains to be further explored to decipher its exact role in aGVHD.

Overall, results from aGVHD mouse models, suggest that Th17 cells may have a role in aGVHD pathophysiology.

Studies in allo-SCT patients

The role of Th17 cells has also been investigated in human aGVHD pathophysiology. Three studies evaluated the relation between the presence of the single-nucleotide polymorphism rs11209026 (1142G>A) in *IL-23R* and aGVHD.[85–87] In two studies,[85,86] there was a significant reduction of aGVHD incidence in patients who were transplanted from a donor with the *IL-23R* single-nucleotide polymorphism, while there was no effect when it was in the recipient, and the third study fail to identify any effect of the polymorphism.[87] In healthy donors, the presence of the *IL-23r* single-nucleotide polymorphism promotes the expression of soluble IL-23R[88] and, consequently, diminished IL-23 signaling, leading to a decreased IL-23-dependent IL-17 and IL-22 production and STAT3 phosphorylation.[89,90] These data suggest that protective effects of the *IL-23R* polymorphism on aGVHD are mediated through selective attenuation of IL-23 induced-Th17 effector function.

Dander *et al.*[91] and Liu *et al.*[92] have reported that Th17 cells and IL-17 serum level were significantly increased in the blood of patients at aGVHD onset, compared with allo-SCT patients without aGVHD. Furthermore, in both studies, the increased number of circulating Th17 cells was accompanied by a decrease in circulating Tregs.[91,92]

Early studies failed to identify Th17 cells infiltrating aGVHD target tissues. Thus, Broady *et al.*[93] reported that only Th1 and not Th17 cells infiltrate the skin of patients with cutaneous aGVHD.

Similarly, Ratajczak *et al.*[94] did not find Th17 cells in skin and gut biopsies of patients with cutaneous or gastrointestinal aGVHD. Identification was based on detection of IL-17+ cells directly by immunohistochemistry in patients' biopsies in both studies,[93,94] or by flow cytometry after *in vitro* culture of dermal cells in the study by Broady *et al.*[93] However, Th17 cells could convert into IFNγ-producing Th1-like cells.[44,45] Given Th17 cells plasticity, IL-17 is probably not the most reliable marker. Therefore, using CD161 and CCR6, two surface marker, of Th17 cells[45,95,96] and RORγt, the key transcription factor that orchestrate Th17 cell differentiation,[14] we have shown that the number of Th17 was significantly increased in the intestinal mucosa and the skin of patients with gastrointestinal or cutaneous aGVHD, compared with allo-SCT patients who did not developed aGVHD.[97,98] Similarly, using the same two markers, van der Waart *et al.*[99] reported that Th17 cells infiltrate aGVHD-affected tissues (intestinal mucosa and skin) while being decreased in the peripheral blood during aGVHD. Recent data have shown that circulating Th17 cells may be increased early after allo-SCT in patients who will develop aGVHD. Thus, Lee *et al.*[100] showed that a high ratio of CD4+ CD161+ to CD8+ CD161+, and an increased level of serum IL-17 at engraftment were associated with subsequent development of aGVHD, and that those CD4+ CD161+ cells expressed high levels of RORγt. Similarly, Betts *et al.*[101] reported that at day 21 after allo-SCT, pSTAT3, a transcription factor that directly drives the transcription of Th17 lineage-specific genes,[15] was significantly increased in CD4+ T cells among patients who will subsequently develop aGVHD. Furthermore they confirmed that the number of CD3+ RORγt+ Th17 cells was significantly increased in aGVHD target tissues.[101] Recently, a novel CD146+CCR5+ T-cell population was identified, this population was significantly increased at gastrointestinal aGVHD onset, and proven to be Th17-related (Li W, Liu L, Gomez A, Zhang Q, Zhang J, Ramadan A *et al.* unpublished data). Moreover, at day 19 post allo-SCT, those cells were significantly increased before aGVHD onset in patients who subsequently developed gastrointestinal aGVHD, suggesting that this CD146+CCR5+ T-cell population could be used as an early biomarker of intestinal aGVHD. Finally, Reinhardt *et al.*[102] demonstrated that peripheral monocytes isolated from patients with skin and/or gastrointestinal aGVHD induce significantly increased level of Th17 cell compared with patients without aGVHD, highlighting that activated monocytes could drive peripheral Th17 cells in aGVHD.

The role of the Th17-related cytokine, IL-22, as in murine models, seems to be dependent upon the cell source. Recently, Munneke *et al.* have shown that appearance of donor origin natural cytotoxicity receptor-positive innate lymphoid cell, an important innate source of IL-22, correlated with a decreased incidence of aGVHD.[103]

Overall, these results suggest that circulating Th17 cells are increased early after allo-SCT in patients who develop aGVHD, and that, at its onset, circulating Th17 decrease in the peripheral because they migrate into the aGVHD target tissue, where they trigger its damage (**Figure 1**).

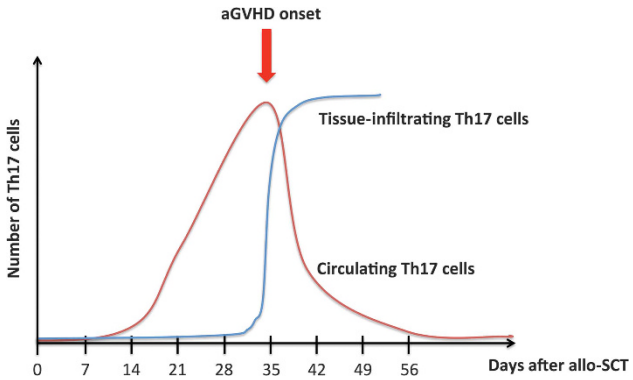

Figure 1 Kinetic of Th17 cells after allogeneic stem cell transplantation in patients who develop acute graft-vs.-host disease. aGVHD, acute graft-vs.-host disease; allo-SCT, allogeneic stem cell transplantation.

Th17 CELLS: A NEW TARGET FOR aGVHD PREVENTION AND TREATMENT

So far, the most widely used immunosuppressive drugs for aGVHD prevention and therapy increase the infection risk, and present side effects other than those related to their immunosuppressive properties. Thus there is a need for more specific and less toxic approaches. Given growing evidence suggesting that Th17 cells have a role in aGVHD, they represent a promising therapeutic target toward which to design new approaches for aGVHD treatment, but also for prevention or pre-emptive therapy, since circulating Th17 are increased before aGVHD onset (**Table 1**, **Figure 2**).

Several monoclonal antibodies, anti-IL-17A (ixekizumab, secukinumab) or anti-Il-17R (brodalumab), have proven to be effective in psoriasis, an IL-17-related autoinflammatory skin diseases.[104–106] However, these results do not guarantee the effectiveness of these monoclonal antibodies in aGVHD. In fact, brodalumab and secukinumab were ineffective for Crohn's disease treatment,[107,108] while IL-17 was reported to drive Crohn's disease.[108] For the IL-22/IL-22R axis, further exploration to delineate its inflammatory vs. protective effects in aGVHD is indispensable, before considering targeting it.

Given IL-1β and IL-6 drive Th17 cell differentiation, therapy targeting IL-1β and IL-6 has been evaluated in clinical trials. Blocking IL-1β using recombinant human IL-1R antagonist was proven to be ineffective for aGVHD prevention,[109] while tocilizumab, an anti-IL-6 receptor monoclonal antibody, has shown promising results for aGVHD prophylaxis in a phase 1/2 trial,[110] and several phase 2 trial are ongoing. As IL-23 expands the pathogenicity of Th17 cells,[27,30,32] it

Table 1 Potential therapeutic agents targeting the Th17 axis for acute GVHD treatment

Target	Drug	Companies	Clinical stage in aGVHD	Clinical trial identifier and publication
Th17 differentiation				
IL-6R	Tocilizumab	Roche	Phase 1/2 completed[110] Phase 1/2 ongoing Phase 2 ongoing Phase 2 ongoing	ACTRN12612000726853 NCT01475162 NCT01757197 NCT02206035
IL-23–p40	Ustekinumab	Janssen	Phase 2 ongoing	NCT01713400
IL-23–p19	Tildrakizumab Guselkumab AMG 139 BI 655066 LY3074828	Merk/Sun Pharma Janssen Amgen Boehringer Ingelheim Eli Lilly	Not evaluated in GVHD	NA
STAT3	Ruxolitinib Tofacitinib	Novartis Pfizer	Retrospective study[116] Not evaluated in GVHD	NA
Th17-related cytokine				
IL-17A	Ixekizumab Secukinumab CNTO 6785 SCH 900117 CJM112	Eli Lilly Novartis Janssen Merk Novartis	Not evaluated in GVHD	NA
IL-17A and IL-17F	Bimekizumab ALX-0761	UCB Merk Serono/Ablynx		NA
IL-17R	Brodalumab	Amgen	Not evaluated in GVHD	NA
IL-21	NNC0114-0005 NNC0114-0006 ATR-107	Novo Nordisk Novo Nordisk Pfizer	Not evaluated in GVHD	NA

Abbreviations: aGVHD, acute graft-vs.-host disease; IL-6R, interleukin-6 receptor; NA, not available; Th17, T helper 17.

appears to be also a promising therapeutic target. Therefore, ustekinumab, a monoclonal antibody that binds the p40 subunit shared by IL-12 and IL-23, approved for psoriasis and effective in Crohn's disease,[111] has demonstrated efficacy in one case report of glucocorticoid-refractory aGVHD.[112] Ustekinumab is currently evaluated for aGVHD prevention in combination with tacrolimus and sirolimus (NCT01713400). Several monoclonal antibodies targeting the IL-23–p19 are also being evaluated in phase 1, 2, or 3 trials for psoriasis and rheumatoid arthritis; raising the possibility to evaluate them for aGVHD treatment. Finally, several monoclonal antibodies targeting IL-21, a cytokine that promotes Th17 cell self-maintenance,[20] are under development for rheumatoid arthritis, Crohn's disease and systemic lupus erythematosus,[113] and represent a potential therapeutic strategy for aGVHD.

Some inhibitory molecules directly target Th17 cells. Thus, pharmacological inhibition with KD025 of Rho-associated kinase 2 significantly diminished STAT3 phosphorylation and binding to *IL-17* and *IL-21* promoters in mouse models.[114] Also, the Janus family kinase (JAK) inhibitors, Tofacitinib, a JAK1/3 inhibitor and Ruxolitinib, a JAK1/2 inhibitor, block STAT3 phosphorylation resulting in the suppression of Th17 cell differentiation.[115] Tofacitinib and Ruxolitinib have proven to be effective for psoriasis treatment in human in phase 3 and 2 trials, respectively. Zeiser *et al.* recently reported a retrospective study evaluating Ruxolitinib for corticosteroid refractory aGVHD in 54 patients.[116] The overall response rate was 81.5%, including 25 complete responses (46.3%), highlighting the therapeutic potential of JAK inhibitors for the treatment of aGVHD.[116] Prospective studies evaluating Ruxolitinib and Tofacitinib for aGVHD prevention or treatment are expected. Finally, several additional molecules that could block the Th17 pathway are at a preclinical development stage, such as inhibitors of RORγt[117] or retinoid acid receptor α agonist.[118]

CONCLUSION AND PERSPECTIVE

Significant achievements have been made in the understanding of Th17 cells pathophysiology. Recent data showing an increased Th17 cell population during or preceding aGVHD are of particular interest, highlighting that these cells could be targeted not only for aGVHD treatment, but also earlier for its prevention. The increased number of monoclonal antibodies and inhibitory molecules targeting the Th17 pathway hold promise for identification of more effective treatment for aGVHD prevention and treatment. Efforts must be pursued to evaluate those forms of treatment in aGVHD.

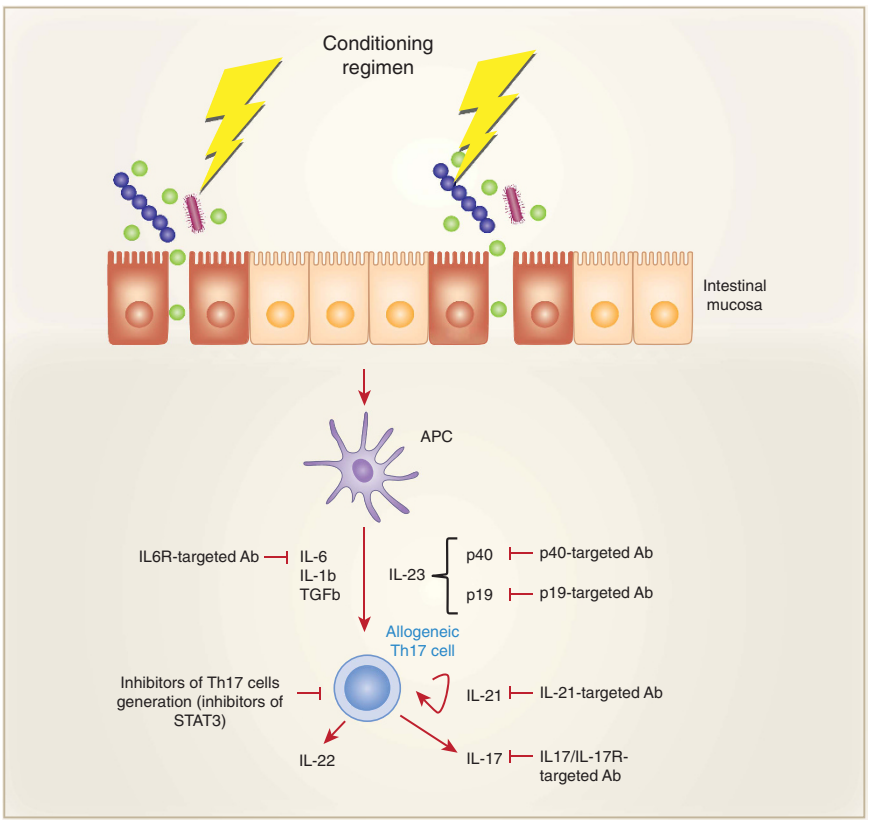

Figure 2 Potential therapeutic targets of the Th17 pathway implicated in acute graft-vs.-host disease, example of the intestinal acute graft-vs.-host disease. The conditioning regimen leads to host intestinal tissue damage and activation of host antigen-presenting cells that will drive Th17 differentiation through IL-6, IL-1β, TGFβ, and IL-23. Various therapeutic tools are available to target Th17 pathway. Cytokines driving Th17 cells differentiation could be target by monoclonal antibodies: tocilizumab target IL-6R ustekinumab target the p40 subunit share by IL-12 and IL-23 and the p19 subunit of IL-23 is targeted by Tildrakizumab, Guselkumab, AMG 139, BI 655066, and LY3074828. Th17 differentiation could also be target by inhibitors of Th17 generation, such as the JAK inhibitors Tofacitinib and Ruxolitinib that block STAT3 phosphorylation. Several monoclonal antibodies could target Th17-related cytokines: IL-17A (Ixekizumab, Secukinumab, CNTO 6785, SCH 900117 and CJM112), IL-17A and IL-17F (Bimekizumab and ALX-0761), and IL-21 (NNC0114-0005, NNC0114-0006 and ATR-107). Alternatively, IL-17R could be targeted by the monoclonal antibody Brodalumab. Finally, given the contradictory data regarding the inflammatory vs. protective effect of IL-22 in aGVHD, no therapeutic strategy related to IL-22 could be proposed at moment. Ab, monoclonal antibody; aGVHD, acute graft-vs.-host disease; APC, antigen-presenting cell; IL-6R, interleukin-6 receptor; TGFβ, transforming growth factor-β; Th17, T helper 17.

ACKNOWLEDGMENTS

FM was supported by educational grants from the "Association for Training, Education and Research in Hematology, Immunology and Transplantation" (ATERHIT). This work was carried out in the context of the IHU-Cesti project, which received French government financial support managed by the National Research Agency, via the "Investment Into The Future" program ANR-10-IBHU-005. The IHU-Cesti project is also supported by Nantes Metropole and the Pays de la Loire Region. We also thank the "Association pour la Recherche sur le Cancer (ARC)", the "Fondation de France", the "Fondation contre la Leucémie", the "Agence de Biomédecine", the "Association Cent pour Sang la Vie", the "Association Laurette Fugain", the "IRGHET" and the "Ligue Contre le Cancer" (Comités Grand-Ouest), for their generous and continuous support to our clinical and basic research work. Our transplant programs are supported by several grants from the French National Cancer Institute. We are grateful to Professor Junia V. Melo (University of Adelaide, Australia, and Imperial College, London) for medical editing of this manuscript.

AUTHOR CONTRIBUTIONS

F.M. designed the manuscript, analyzed the literature, wrote and commented on the manuscript. B.G. analyzed the literature, assisted in writing, and commented on the manuscript. B.L. analyzed the literature, assisted in writing, and commented on the manuscript. M.M. designed the manuscript, analyzed the literature, wrote and commented on the manuscript. All authors approved submission of the manuscript for publication purposes.

DISCLOSURE

The authors declare no conflict of interest.

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