Effector CD4+ T cells, the cytokines they generate, and graft-versus-host disease: something old and something new

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Running Title: T cell lineages and GvHD
Abstract:

Graft-versus-host disease (GvHD) is a syndrome that results from minor and major MHC incompatibilities between the donor and recipient. More than fifty years after its initial description, the pathophysiology of GvHD remains poorly understood. Nonetheless, donor T cells have been shown to be critical to the pathophysiology of acute and chronic GvHD, yet precisely how they function remains unclear. The effector mechanisms by which donor T cells mediate tissue inflammation is even less well understood. Identification of several new lineages of CD4⁺ T cells made in the last decade and their roles in the pathophysiology of T cell-mediated diseases has shed new light on these effector mechanisms. In this review, we summarize the recent descriptions of these T cell lineages, and the current data supporting their role in acute and to a lesser extent chronic GvHD. Investigations into the activity of these new T cell lineages may provide more rationale approaches to the treatment and/or prevention of GvHD.
Introduction:

The first descriptions of acute graft-versus-host disease (GvHD) came after investigations into the capacity of radiation to ablate endogenous bone marrow function in the 1950s. When mice were treated in this manner and given splenocytes from a non-congenic donor strain to facilitate the regeneration of blood cells, an illness characterized by progressive weight loss, hunched posture, decreased activity and diarrhea was noted\textsuperscript{1}. This was initially termed “secondary disease” or “runting” to differentiate it from the initial toxicity of the conditioning regimen and its resulting aplasia. Subsequent work indicated that donor T cells that recognize minor or major histocompatibility differences in the host were critical mediators of GvHD\textsuperscript{2-4}.

Thus, a role for donor T cells in the pathogenesis of GvHD has been clear for over 30 years. However, the mechanism by which T cells mediate this process is not completely understood. This review will focus on the multiple different new subsets of effector CD4\textsuperscript{+} T cells that have been recently described, and their role in acute and chronic GvHD. We will not focus on regulatory T cells (e.g. T\textsubscript{reg})\textsuperscript{5,6} or T regulatory type 1 (Tr1) cells,\textsuperscript{7} or provide a comprehensive overview of each lineage both of which have been recently reviewed\textsuperscript{8-12}.

Th1/Th2 Cells:

In 1986, Mosmann and Coffman published seminal work on the clonal behavior of T cells. From a large panel of antigen-specific and autoreactive T cell clones, they were able to divide these cells into two distinct groups\textsuperscript{13}. T-helper type 1 (Th1) clones generated IL-2, IL-3 and IFN-\textgreek{\gamma} while T-helper type 2 (Th2) cells generated IL-3, TCGF2 and MCGF2. Subsequent work by Cherwinski \textit{et al.} demonstrated that Th1 clones generated IL-2, GM-CSF and IFN-\textgreek{\gamma},...
while Th2 clones generated IL-4 and IL-5. Further, Stevens et al. showed that both types of T cells induced the generation of IgM and IgG3 by B cells. However, Th1 cells specifically induced IgG2a, while Th2 cells induced the production of IgG1 by B cells. Multiple investigators showed that the Th1 and Th2 lineages were fixed and separate as they were not able to generate Th1 clones from polarized Th2 cells and vice versa. Eventually, this became the accepted paradigm, with studies demonstrating that Th1 cells played a critical role in the clearance of intracellular organisms, most typically viral pathogens, whereas Th2 responses were critical for the response to parasitic infections. Additionally, each of these lineages was associated with specific and distinct autoimmune diseases.

Differentiation of CD4+ T cells by the proteins or mRNA they generated remained inexact, with overlapping activity found for Th1 and Th2 cells. However, critically important work from the laboratories of Glimcher and Flavell identified lineage specific transcription factors for Th1 and Th2 cells. T-BET (TBOX21) was found to be the critical transcription factor for the generation of Th1 cells, while GATA-3 served that role for Th2 cells (see Fig 1).

Until ten years ago, Th1 and Th2 T cells were the only truly separate CD4+ lineages. Although groups of T cells such as T-regulatory type 1 (Tr1) or T-helper type 3 (Th3) cells were described, it was not clear that these cells were separate lineages. Thus, much of the work inferring a role for Th1 or Th2 cells in the biology of GvHD has been done with the assumption that Th1 or Th2 cells were the sole T cell lineages. In this review, we will summarize the data suggesting that other CD4+ T cell lineages also are critical to the pathophysiology of GvHD.

**Th17 cells**
The initial description of a subset of CD4$^+$ T cells separate from Th1 cells that mediated inflammation came from the interesting finding that mice lacking the IL-12 p40 chain behaved differently than mice lacking the IL-12 p35 chain. This conundrum was explained when work demonstrated that p40 could pair with a second chain, p19, to generate the cytokine IL-23. This cytokine was shown to be important for the generation of T cells producing IL-17A. However, whether T cells producing IL-17A differed from Th1 cells was not clear, until two seminal papers clearly established the Th17 lineage as independent and distinct$^{22,23}$.

Early work suggested a critical role for IL-23 in the development of Th17 cells from naïve T cells ($T_{ns}$) (Fig 1). However, the initial polarization of $T_{ns}$ was found to depend critically on the presence of transforming growth factor beta (TGF-β), a cytokine that had previously been shown to inhibit both Th1$^{24}$ and Th2 development$^{25}$. At least one other pro-inflammatory cytokine must be combined with TGF-β to effectively induce Th17 cells$^{26-32}$. In mice, IL-6 is the critical second mediator$^{26,27}$, and also appears to be required for their normal development in vivo$^{33}$. In humans, the nature of this second signal is less clear, with IL-6, IL-21, IL-23, and IL-1β all being implicated$^{29-32}$.

In mice, Th17 polarization proceeds upon T cell receptor (TCR) and CD28 engagement in the absence of Th1 and Th2 polarizing cytokines in the presence of both TGF-β and IL-6. The latter has been shown to directly activate the transcription factor STAT-3, which is required for Th17 differentiation$^{34}$ (Fig 1). Full expression of the Th17 phenotype depends on the orphan nuclear receptor, RORγt. RORγt induces the expression of IL-17A and IL-17F$^{33}$, contributes to the generation of IL-23R$^{35,36}$, and mediates IL-22 production$^{37}$. Recently, a second transcription factor, RORα, has been found to respond to STAT-3, and has several overlapping functions with RORγt including the transcription of IL-17A and to a lesser extent IL-17F.
IFN-\(\gamma\) is the prototypical Th1 cytokine, driving initial Th1 polarization and serving as their principal inflammatory mediator. It is becoming increasingly clear, however, that equating IFN-\(\gamma\) production with Th1 cells is an oversimplification, as other cell types including Th17 cells are able to produce abundant IFN-\(\gamma\) under certain conditions. Several groups including our own have reported that a substantial fraction of IL-17A producing Th17 cells co-express IFN-\(\gamma\) both \textit{in vitro} and \textit{in vivo}\textsuperscript{38-42}. In addition, recent data have demonstrated that the Th17 lineage is not stable. Lee \textit{et al.}\textsuperscript{43} and Mukasea \textit{et al.}\textsuperscript{44} found that Th17 cells cultured in the presence of IL-12 with or without exogenous TGF-\(\beta\) rapidly extinguished IL-17F and ROR\(\gamma\)t expression, and upregulated IFN-\(\gamma\) via signaling through STAT-4 and T-bet, consistent with skewing toward the Th1 lineage. Our group and others\textsuperscript{45,46} found similarly that almost a pure population of Th17 cells extinguished production of IL-17A and IL-17F if cultured in the presence of IL-12. Especially relevant for GvHD pathophysiology, this process occurs more readily in a lymphopenic environment\textsuperscript{47}.

The previous studies and data demonstrating that inducible T\(_{\text{reg}}\)s can convert under certain conditions to Th17 cells and vice versa have led investigators to evaluate the mechanism for this finding\textsuperscript{10}. Studies have shown substantial epigenetic modifications for lineage changes by T cells. Several recent studies have shown that chromatin alterations at critical lineage-specific loci such as \textit{FOXP3}and/or \textit{RORC} are critical in these changes, and suggest that the notions regarding lineage fixation by T cells is overly simplistic\textsuperscript{48}.

\textbf{T Follicular Helper Cells (Tfh):}

Previous investigators demonstrated that antibody generation against pathogens and critically immunoglobulin class switching required the interaction of CD4\(^+\) T cells with B cells.
As IL-4 could mediate much of this effect, the presumption had been that this process was mediated by Th2 cells. However, in 2002, a third effector T cell lineage, T follicular helper (Tfh) cells, was described simultaneously by Breitfield et al. and Schaeerli et al. Tfh cells express the chemokine receptor, CXCR5, which is critical for their migration into the germinal center where antibody generation and class switching occur. IgG and IgA generation by B cells was significantly increased in the presence of Tfh. Chtanova et al using gene chip analysis demonstrated that Tfh cells express a distinct transcription profile characterized by the differential expression of a number of genes including: BCL6, IL6R, CD30l, CD27, CD84, CD200, and IL21 in addition to CXCR5 and IL4 when compared to Th1, Th2, central and effector memory cells.

Tfh cells migrate from the blood into the B cell follicle where they reach B cell-rich areas. The mechanism by which exposure to antigen programs cells to become Tfh is not entirely clear, although TCR affinity for class II MHC/peptide appears to be important as Tfh have higher affinity TCRs. The transcriptional repressor, BCL-6, is critical for Tfh activity as forced expression in T cells generates features consistent with Tfh cells, and perhaps most importantly, its absence leads to an inability to generate Tfh cells and as a consequence complete loss of germinal center B cells and the germinal center reaction. Sites for the binding of BCL-6 were found in the promoters of TBOX21 and RORC. Moreover, forced expression of BCL-6 diminished the expression of TBOX21 and GATA-3, suggesting that T cells undergoing the program to generate Tfh cells suppress transcription factors critical for Th1 and/or Th2 polarization.

Th22 Cells:
IL-22 is a cytokine that is a member of the IL-10 superfamily and signals via the IL-22 receptor composed of the common IL-10R2 and specific IL-22Rα19. The function of IL-22 is complex, as it has been demonstrated to mediate both pro and anti-inflammatory activity. IL-22 was initially shown to be produced by Th17 cells and the expansion of cells generating this dependent on IL-2353. IL-22 in conjunction with IL-17A and/or IL-17F is important in the generation of small antimicrobial peptides important for the health of the skin.

Eyerich et al were the first to describe a specific cellular T cell lineage for the production of IL-2254. They evaluated human T cells from patients with psoriasis, atopic eczema and allergic contact dermatitis. A subset of T cells secreting IL-22 alone was found primarily in the blood and skin of patients with atopic eczema. Th22 clones generated IL-22 and in addition IL-10 and/or TNF. Sorting for the expression of the skin homing chemokine receptor, CCR10, enriched for IL-22-expressing memory T cells. Transcriptome analysis indicated that Th22 cells generated CCL7, CCL15 and, unlike Th17 cells, did not upregulate CCL20. The transcription factors BNC2 and FOXO4 were overexpressed by Th22 cell clones, while there was reduced expression of RORC, GATA3 and TBOX21 compared to Th1, Th2 or Th17 cells. A second group used expression of the chemokine receptors CCR4, CCR6, and in particular CCR10 to characterize human CD4+ T cells55. Transcription factors critical for T cell polarization could be differentiated based on the expression of CCR10. CCR10+ T cells expressed the aryl hydrocarbon receptor (AHR), while CCR10− T cells expressed RORC. Knocking down RORC inhibited the production of IL-17A and IL-22 by T cells, while targeting AHR specifically inhibited the production of IL-22. This group’s Th22 clones generated IL-22, IL-13 and TNF. Subsequent work suggested that Langerhan’s cells, a specific type of skin DC, can stimulate
Th22 cells independent of Th1 and/or Th17 activity\textsuperscript{56}. At this time, a specific Th22 lineage has yet to be described in mice.

**Th9 Cells:**

Interleukin-9 (IL-9), a cytokine traditionally associated with the Th2 lineage, has been implicated in immunity to helminths, allergic responses, and the expansion of B cells. More recently, IL-9 expression has been linked to other T cell subtypes, including Th17 cells and inducible T\textsubscript{regs}, and shown to mediate both pro-inflammatory and immunomodulatory effects \textit{in vivo}\textsuperscript{57,58}. Data are now emerging to suggest that IL-9 producing T cells may represent another, distinct Th subset. Th9 cells have been shown by two groups to generate abundant IL-9 and IL-10, with minimal expression of IL-4, IL-5, IL-13, IFN-\gamma, or TNF\textsuperscript{59,60}. Further, these cells were not found to express any of the known T helper transcription factors that define the Th1, Th2, iT\textsubscript{reg} or Th17 lineages (\textit{TBOX21, GATA3, FOXP3}, or \textit{RORC} respectively). Th9 cells could be generated \textit{de novo} from naïve CD4\textsuperscript{+}CD25\textsuperscript{−} cells under unique polarizing conditions that included exogeneous IL-4 in the presence of TGF-\beta. In addition, committed Th2 cells could be “reprogrammed” to a Th9 phenotype upon re-activation in the presence of TGF-\beta alone, with extinction of \textit{GATA3} and \textit{IL4} expression\textsuperscript{59}. These Th2 precursors could not be polarized back to Th1 cells despite repeated stimulation in the presence of IL-12, suggesting that Th9 transition may represent a unique plasticity for the Th2 lineage.

At the present time, the normal tissue distribution and immune function of these cells is poorly understood. In general, and with the exception of IL-9 producing T\textsubscript{regs}, Th9 cells appear to be pro-inflammatory despite their abundant IL-10 production, and have been shown to exacerbate intestinal inflammation in T cell adoptive transfer colitis models\textsuperscript{60}. Interestingly,
these cells may also possess a strong tropism for nervous tissue, as they are able to induce severe peripheral neuritis in murine recipients. Th9 cells, unlike Tr1 cells, which also produce abundant IL-10, do not exhibit any apparent immunosuppressive properties in vitro, and appear to proliferate normally in response to anti-CD3 stimulation.

**T Cell Subsets and GvHD: Preclinical Evaluations:**

**Th1 T cells:** In most murine models, CD4+ T cells are critical to the induction of GvHD either by the need for CD4+ T cells to produce IL-2, which mediates robust allospecific CD8+ T cell proliferation or by the generation of effector proteins like TNF or cytolytic activity mediated by FAS/FASL. Early studies demonstrated significant expression of IFN-γ in GvHD target organs, suggesting that Th1 cells were critical mediators of tissue pathology. From these findings, investigators hypothesized that blocking the function of the Th1 effector cytokine, IFN-γ, early post stem cell transplant (SCT) would diminish or perhaps prevent acute GvHD. Interestingly, this was not found. Our group evaluated whether donor T cells incapable of generating IFN-γ could mediate GvHD in a murine model. The median survival for recipient mice given IFN-γ knockout (ko) donor cells was 21 days compared to 38 days for the use of wild type (WT) T cells. We demonstrated a similar effect using anti-IFN-γ mAb. These data suggested that not only was IFN-γ not critical for the generation of acute GvHD, but that its absence exacerbated GvHD lethality. Subsequent studies demonstrated that donor-derived IFN-γ production worsened gastrointestinal GvHD but reduced lung injury. Interestingly, our group found that IFN-γ, while is critical for GI tract pathology, also mediated the induction of indolamine 2-3 dioxygenase, which catabolizes the critical amino acid tryptophan to kynurenine and blocks T
cell proliferation in the GI tract\textsuperscript{64,65}. Thus, IFN-\(\gamma\) may mediate separate functions at the same target organ during GvHD.

A second approach to evaluate the function of different T cell subsets in GvHD focused on the signaling proteins critical to the generation of IFN-\(\gamma\) and as a result Th1 cells.\textsuperscript{66} Nikolic \textit{et al.} evaluated the induction of acute GvHD using donors unable to generate \textit{STAT4} (Th1 impaired) or \textit{STAT6} (Th2 impaired)\textsuperscript{67}. They found that \textit{STAT4} ko donor T cells mediated acute GvHD with delayed kinetics compared to WT or \textit{STAT6} ko T cells. Interestingly, recipients of \textit{STAT4} ko cells demonstrated substantial liver pathology with hepatocellular necrosis, and extensive cutaneous changes with marked dermal infiltration and lysis along the epidermal-dermal junction, similar to that recently described after the infusion of Th17 cells generated \textit{ex vivo}\textsuperscript{38}. This severe skin pathology was not found using \textit{STAT6} ko donors. Recipient mice given \textit{STAT6} ko donor T cells had earlier and more severe clinical GvHD, and manifested profound inflammation in the colon with little involvement of the skin or liver. These data have suggested that Th1 cells play a critical role in tissue inflammation in the GI tract (Fig 2).

**Th2 Cells**: Initial studies demonstrated that Th2 cells, when added to donor splenocytes, could mitigate acute GvHD with significant attenuation of GvHD pathology in the GI tract and liver\textsuperscript{68,69}. Follow-up studies demonstrated that the release of proinflammatory cytokines induced by lipopolysaccharide was reduced when Th2 cells were added to the donor inoculums\textsuperscript{70}, suggesting that Th2 cells could be therapeutically beneficial. Quite recently, two groups have sought to evaluate the role of Th2 cells in acute GvHD using genetic approaches\textsuperscript{71,72}. Tawara \textit{et al.} used T cells from mice in which the \textit{IL4/IL5/IL13} gene cluster was targeted by homologous recombination and crossed to \textit{IL9} ko mice. In a complete MHC mismatched model, they found enhanced GvHD which correlated with increased donor T cell proliferation, production of TNF.
in vivo and T cell production of IL-2, IFN-γ and IL-17A in vitro. Yi et al used a similar approach by infusing donor T cells unable to generate IFN-γ, IL-17A or IL-4. Absence of IFN-γ and IL-17 led to diminished inflammation in the colon, liver and skin but very modestly increased inflammation in the lung. Using an anti-IL-4 antibody, they found reduced lung pathology scores, suggesting a role for IL-4 generation in idiopathic pneumonia syndrome (IPS)/GvHD in the lung (Fig 2). They did not find GvHD in any other target organs in the absence of IL-17 and IFN-γ, suggesting that Th2 cells may not play a critical role in the pathophysiology of GvHD in the colon, liver, skin or small bowel in animal models. These and additional studies indicate that Th1 cells are critical for acute GvHD pathology in the GI tract, but that IFN-γ itself, has detrimental and beneficial effects that are organ system dependent. A specific role for Th2 cells themselves could not be found, with the exception of IPS post SCT. A role for Th2 cells was inferred from the pathology found using donor mice unable to polarize to a Th1 response (STAT4 ko), which may be complicated by the ability of other T cell lineages such as Th17 cells to mediate these effects.

Chronic GvHD: Our understanding of the function of T cells in cGvHD is less advanced compared to that in acute GvHD partly due to the difficulty with generating animal models that duplicate clinical disease. Organs such as the kidney, which is rarely involved clinically, are often a significant site of pathology in cGvHD murine models. Nevertheless, studies have suggested in both non conditioned F1 recipients using donor T cells alone, and in minor mismatched models with sublethal irradiation, that Th1 polarization can reduce tissue pathology, while Th2 polarization exacerbated this.

Th17 Cells
Th17 cells generate the proinflammatory cytokines IL-17A, IL-17F, IL-21, IL-22 and TNF, and their function is mediated by the transcription factors RORγt and RORα (Fig 1). The contribution of Th17 cells to GvHD pathology in mice is controversial. Our group addressed this issue by generating highly pure B6 (H-2b) Th17 cells in vitro, and administering them alone or combined with B6 Tns to haploidentical B6D2 F1 (H-2bd) recipients. Infusion of Th17 cells resulted in aggressive, lethal GvHD, with unusually severe pathology observed in the skin and lungs38 (Fig 3). These effects depended partly on IL-17A, as neutralization with an anti-IL-17A antibody greatly ameliorated skin disease, while blocking TNF ameliorated systemic GvHD. Further, these effects were independent of IFN-γ, as Th17 cells generated from IFNG ko B6 animals produced nearly identical results. Hill et al. suggested a prominent role for Th17 cells in cutaneous GvHD77. The authors demonstrated that donor mice undergoing stem cell mobilization with human g-CSF show an increased proportion of CD4+ and CD8+ IL-17A producing T cells within their spleens in an IL-21-dependent manner. When g-CSF mobilized splenocytes were subsequently transferred into irradiated recipients, sclerodermatous skin lesions developed which depended critically on the production of IL-17A (Fig 3).

However, it has been harder to demonstrate a conclusive role for the cytokine, IL-17A, in GvHD induced by Tns. Kappel et al.40 found that whole T cells from IL17a ko B6 animals generated GvHD responses that were essentially identical to those induced by WT cells, although, when purified CD4+ IL17a ko T cells were utilized, there was improved transplant outcomes with recipients demonstrating a prolonged median survival time. Overall survival was unaffected, however, leading the authors to conclude that while IL-17A contributes to the early development of CD4+ T cell-mediated inflammation, it is dispensable for GvHD as a whole.
Substantially different results were obtained by Yi et al using a similar model\textsuperscript{78}. \textit{IL17a} ko splenocytes and whole T cells were found to exacerbate GvHD and to accelerate recipient mortality compared to WT cells. The inferior outcomes seen with \textit{IL17a} ko cells could be prevented by the administration of exogeneous IL-17A or neutralizing anti-IFN-\(\gamma\), leading the authors to conclude that in the absence of IL-17A, Th1 differentiation was enhanced, which has been found in other models\textsuperscript{79}. As the only differences in the experimental systems that generated these disparate results was the use of a lower dose of radiation it is possible that the function of IL-17A post transplant is timing or conditioning dependent, or affected by the microbiota in the different investigator’s colonies\textsuperscript{80}.

Given the plasticity of the Th17 lineage\textsuperscript{10} and its numerous inflammatory mediators, the most straightforward approach to addressing the contribution of Th17 cells to GvHD pathogenesis is to use donor immune cells lacking ROR\(\gamma\)t, which would be unable to generate Th17 cells\textsuperscript{33}. In work by Iclozan et al., the authors transferred WT or ROR\(\gamma\)t ko purified CD4\textsuperscript{+} B6 T cells to lethally irradiated Balb/c mice, and observed nearly identical overall survival percentages, median survival times, and percent weight loss between the two treatment groups\textsuperscript{39}. This led the authors to conclude that “Th17 cells are sufficient but not necessary to induce GVHD.” Although the authors transferred between 1-2x10\textsuperscript{6} CD4\textsuperscript{+} cells per recipient, uniform lethality was not seen in either group as late as transplant day +50. Our own group has conducted similar experiments with WT B6 versus ROR\(\gamma\) ko (lacking both isoforms ROR\(\gamma\) and ROR\(\gamma\)t) B6 donor animals, using both Balb/c and B6D2 F1 mice as recipients. In both systems using a lower dose of donor T cells, ROR\(\gamma\) ko cells generated greatly attenuated GvHD and prolonged recipient survival, although this was less profound in the complete MHC mismatch model\textsuperscript{81}, suggesting that targeting both isoforms of ROR\(\gamma\) substantially impacted on acute
GvHD. The reasons for the apparent discordant results with Iclozan et al. are unclear, but may relate either to the dose of T cells used, the difference in the infusion of whole versus CD4+ T cells or whether the knockout targets the entire RORC locus or specifically RORγt.

Recent work in which alloreactive T cells are transferred into RAG ko syngeneic hosts to generate a model of chronic GvHD alloreactivity leading to autoreactivity found autoimmune damage was not modified by blocking IL-17A genetically or using mAb therapy75. Whether other cytokines generated by Th17 cells, such as IL-21 which plays a critical role in antigen-specific B cell function, are important in chronic GvHD is not yet known76.

**T Cell Subsets and GvHD: Clinical Evaluations**

Much of the information regarding the role of T cell subsets in clinical GvHD is confusing and contradictory, and comes from the presence of specific cytokines in the serum of GvHD patients83-85, in lesional tissue biopsies83, 89 or is inferred from single nucleotide polymorphisms present in cytokines genes (SNPs)87-88. Interestingly despite the perceived skewing of GvHD toward a Th1 process, multiple investigators have not found increased serum levels of IL-12 after allo-SCT compared to healthy donors82. Consistently, investigators have found increased protein or mRNA for IL-6, TNF and IL-1β, which in one study correlated with disease severity83-85 and in another with disease onset86. Interestingly, these cytokines would polarize toward a Th17 response. Th17 cells have been found increased by ELISPOT and intracellular cytokine staining in the peripheral blood of patients with acute and chronic GvHD, and this correlated in one study with active disease41. SNPs in the regulatory region of IFN-γ leading to increased IFN-γ expression have been associated with an increased incidence of acute
GvHD but only when present in the recipient and not the donor. Thus, serum evaluations would support indirectly a role for Th17 cells in the pathophysiology of acute GvHD.

At lesional sites, we found increased expression of IL2, IL4 and IFNg mRNA from skin biopsy samples in patients with GvHD suggesting a mixed T cell response. A number of investigators have noted increased expression of TNF in the skin of patients with acute GvHD, with one group correlating this with CCR10-infiltrating T cells. This corresponds to the 70% response rate at this site using the TNF-targeted therapy infliximab. Dander et al. noted increased numbers of Th17 cells in the skin of patients with active chronic GVHD compared to control samples collected from skin cancer patients, with most of these IL-17A-producing cells coexpressing IFN-γ. Somewhat conflicting results were obtained in a larger series from Broady et al. Little to no IL-17 or IL-22 was found from ex vivo stimulated T cells isolated from the skin of patients with GvHD compared to healthy controls or samples obtained from psoriasis patients. A third series examined Th17 involvement in human cutaneous GvHD. Skin biopsies were obtained from patients with either acute or chronic lichenoid cutaneous GvHD, and skin infiltrating Th17 cells identified on the basis of their expression of IL-17A by immunohistochemistry. At the same time, infiltrating Tregs were quantitated using Foxp3 staining. The investigators demonstrated that the Th17/Treg ratio was significantly lower than that seen in their non-GvHD controls, which argued against a pathogeneic role for the Th17 subset.

Thus, at this time there are data to support involvement of Th1 and Th17 cells in skin GvHD with perhaps a greater involvement of Th17 cells in chronic GvHD of the skin. The reasons for the differences between these clinical studies and the preclinical data are not entirely clear. One concern is that calcineurin inhibitors block the production of IL-17A, which may lead
to an underestimation of Th17 cells if they are only identified according to their production of IL-17A. A second issue would be the plasticity of Th17 cells, which may preclude longitudinal assessment of these cells *ex vivo* in the absence of polarizing cytokines. The timing of these evaluations may be critical as Th17 cells may readily convert to Th1 cells after the cytokine storm induced by conditioning has abated.

The overwhelming preponderance of preclinical data suggest that Th1 cells are critical mediators of tissue pathology in the GI tract. The little human data that does exist has essentially yielded similar results to the animal studies, with little evidence for a robust Th17 response within gastrointestinal biopsies taken from GvHD patients. Tissue inflammation in the liver post SCT has been harder to define. Dander *et al.* found dual IL-17A, IFN-γ-expressing T cells in the liver in patients with active cGvHD, suggesting a role for Th17 cells skewed toward a Th1 response. Interestingly, the efficacy of infliximab is poorest for treating patients with GvHD involving the liver, suggesting that TNF may not be a critical mediator from Th17 cells at this site.

**Other Lineages:**

While there are no current data regarding the function of Th22 cells in GvHD, the activity of IL-22 makes it an interesting target for patients with cGvHD (Fig 3). Our group has very preliminary data suggesting substantial generation of mRNA for *IL22* in the skin of patients with cGvHD (Coghill and Serody unpublished). As IL-22 appears to be critical for keratinocyte proliferation and acanthosis in an inflammatory setting and epidermal thickening one of the hallmarks of cGvHD is markedly induced byIL-22, IL-22 production by Th22 or Th17 cells may play an important and targetable role in skin pathology during cGvHD. In addition to
alloantibody and autoantibody responses\textsuperscript{92-94}, a coordinated T-B response to minor histocompatibility antigens has been well described in human chronic GvHD\textsuperscript{95}. A role for Tfhs in autoimmune diseases has been described,\textsuperscript{96,97} and suggests that further investigation of these cells in GvHD pathogenesis will define further a role for Tfhs and B cells in chronic GvHD.

What role if any Th9 cells play in the pathogenesis of GvHD remains purely speculative. In solid organ transplant models, IL-9 expression by inducible T\textsubscript{regs} has been shown to promote graft tolerance through the recruitment of immunosuppressive mast cells\textsuperscript{58}. This would suggest a possible protective role for the cytokine in the HSCT setting. Conversely, Th9 cells might worsen acute GvHD by directly exacerbating intestinal injury, or could potentiate cGvHD responses by promoting pathogenic B cell expansion and/or autoantibody production. Future work should evaluate for the presence of these cells especially in the GI tract in animal models and clinical samples.

Summary

Since the initial description of Th1 and Th2 cells, there has been a substantial increase in our understanding of T cell fate determination. In this review, we’ve provided a framework for understanding these new lineages as they impact on acute or chronic GvHD. Current data would suggest that Th1 cells play a critical role in the pathophysiology of acute GvHD with a substantial amount of data suggesting that GI tract involvement is mediated in part or in total by Th1 cells. There is little pre-clinical or clinical information to implicate Th2 cells in acute GvHD globally or at a specific site with the possible exception of the lung. Th2 cells may be critical however in chronic GvHD. The effector lymphocytes that mediate GvHD in the skin and liver are less clear although pre-clinical and some but not all clinical data suggest a role for Th17
and/or Th1 cells. Additionally, T cell lineage commitment is much more dynamic than previously believed, and that it may be more appropriate to evaluate inflammatory nodes such as a combined Th1/Th17/Th22 axis for acute GvHD, or a combined Th2/Tfh/Th9 axis for cGvHD. Recent work would support this approach. Over the past decade much new has been learned—the time has come to get past the Th1/Th2 paradigm and to address the complexity and plasticity of T cell lineage commitment as a new approach for the treatment/prevention of GvHD.
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Authorship:

J.M.C. assisted in writing the manuscript, S.S. assisted in writing the manuscript, T.P.M. assisted in writing the manuscript and generated the figures, W.J.M. edited the manuscript, B.R.B. assisted in writing and editing the manuscript, J.S.S. conceived of the project, assisted in writing and editing the manuscript.

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80. Kuroda R, Nishimura R, Sato K, et al. Role of IL-17 varies at different periods after hematopoietic stem cell transplantation: proection from acute graft-versus-host disease and


Figure Legends

**Figure 1. CD4+ T cell effector subsets.** Antigen-specific stimulation of naïve CD4+ T cells in the presence of certain cytokines induces expression of lineage-specific transcription factors, resulting in differentiation into CD4+ T cell effector subsets. IFN-γ and IL-12 lead to the expression of T-bet, resulting in Th1 cell differentiation. CD4+ T cell activation in the presence of IL-4 results in Th2 cell development mediated by GATA3; however, the addition of TGF-β causes differentiation into Th9 cells. The combination of IL-6 and TGF-β is necessary for RORγt expression, resulting in Th17 cell development; whereas the presence of IL-6 alone or with other unknown cytokines possibly results in Th22 cell differentiation via expression of AHR. Finally, IL-21 appears to be important for the development of Tfh cells through induction of Bcl-6.

**Figure 2. Th1 and Th2 cells in GVHD.** Th1 cells play a significant role in GvHD pathogenesis in the gastrointestinal (GI) tract. Donor-specific Th1 cells migrate to the GI tract and liver via the chemokine receptors CCR9 and CCR5. During pre-transplant conditioning, the integrity of the epithelial barrier is compromised, resulting in translocation of bacterial products and activation of local antigen presenting cells (APC). These activated APCs secrete IL-12, which is necessary for Th1 cell development and expansion. Th1 cells secrete IFN-γ, which has dual roles in the GI tract. IFN-γ and TNF facilitate further Th1 cell development and activation of allospecific cytotoxic T lymphocytes (CTL), resulting in tissue damage. Conversely, Th1-derived IFN-γ can induce the immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO) in APCs, causing T cell anergy and apoptosis. Th2 cells migrate to the lung via CCR4, where they secrete IL-4, IL-5 and IL-13. IL-4 and IL-13 act upon lung epithelium, causing inflammation and tissue remodeling that ultimately leads to pulmonary fibrosis. IL-5 facilitates expansion of eosinophils (Eos) that are recruited to the lung by CCL11, which can further exacerbate lung tissue damage.
Figure 3. Th17 cells in GVHD. Th17 cells traffic to the lung and skin via CCR6, where they mediate tissue damage. In the lung, Th17 cells secrete IL-17A, IL-17F and TNF, which induce secretion of proinflammatory cytokines and chemokines by epithelial cells. Th17-derived cytokines act directly on neutrophils (neut), resulting in production of matrix metalloproteinases (MMPs), reactive oxygen species (ROS) and TNF. In the skin, IL-17A and IL-17F produced by infiltrating Th17 cells cause the production of several proinflammatory cytokines and chemokines by keratinocytes, resulting in further leukocyte recruitment and local tissue damage. IL-22 is secreted by Th17 cells and possibly Th22 cells activated by Langerhan’s cells (LC). IL-22 induces keratinocyte proliferation, resulting in acanthosis and cutaneous GvHD pathology.
Figure 3

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Effector CD4+ T cells, the cytokines they generate, and graft-versus-host disease: something old and something new

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