Dig Dis Sci (2017) 62:2208–2210
DOI 10.1007/s10620-017-4655-2

EDITORIAL

# CD4 T Cells in IBD: Crossing the Line?

**Elisa K. Boden**[1] · **James D. Lord**[1,2]

Received: 13 June 2017 / Accepted: 15 June 2017 / Published online: 23 June 2017


Several lines of evidence have suggested a major contribution of CD4+ T cells toward the pathogenesis of inflammatory bowel disease (IBD). A growing body of genetic associations in Crohn's disease (CD) and ulcerative colitis (UC) have identified polymorphisms in or near genes central to CD4+ T cell function, such as the receptor for interleukin (IL)-23 [1], a cytokine essential for the survival of the Th17 subset of CD4+ T cells, which is implicated in the pathogenesis of several animal models of autoimmunity [2]. Similarly, clinical efficacy has been conclusively demonstrated in large clinical trials of drugs targeting proteins important for CD4+ T cells. Finally, IBD patients infected with human immunodeficiency virus (HIV) have a milder course with fewer disease flares likely as a consequence of CD4+ T cell depletion [3]. Yet, the mechanism by which CD4+ T cells contribute to IBD pathogenesis has remained elusive.

CD4+ T cells have historically been classified into functional subsets according to their pattern of cytokine secretion. In the 1980s, T cell subsets were described as either Th1 cells that secrete interferon (IFN)-γ, important for clearance of intracellular pathogens or Th2 cells that secrete of IL-4, essential to the response to extracellular parasites. However, as new subsets of T cells have been identified, the known system of T helper subsets has become increasingly complex, now including Th17 cells, Th9, T follicular helper ($T_{FH}$) cells, and FoxP3+ regulatory T cells (Tregs). During T cell development, environmental cues induce the expression of "master" transcription factors that define the T cell lineages and direct the expression of genes (chemokine receptors and cytokines) important for the T helper subset role in host–pathogen defense (Fig. 1). The intestinal microenvironment requires a unique balance of effector T cells armed to fight potential pathogens and FoxP3 expressing Tregs, which turn down the immune response to dietary and commensal antigens.

In this month's issue of *Digestive Diseases and Sciences*, Li et al. [4] report on the presence of CD4+ T cells in the intestinal mucosa which blur conventional lines by which T cell subsets have been defined. This study expands upon existing reports by the authors [5] and others [6] showing that IBD patients more than healthy subjects harbor T cells that express the Treg marker FOXP3, and yet are able to produce IL-17 upon activation, or express the nuclear transcription factor RORγt, similar to Th17 cells [2]. Similar to prior studies [5, 6], the group reported a trend towards these Treg/Th17 crossover cells being more prevalent in IBD subjects with higher clinical activity scores. IBD patients also had an increased percentage of FoxP3+ Tbet+ cells in the lamina propria (LP), representing Treg/Th1 crossover T cells. Furthermore, the authors found that UC but not CD patients have increased numbers of FoxP3+ Tregs with features of Th2 cells, such as IL-13 production or GATA3 expression. While the percentage of CD4+ T cells expressing both FoxP3 and IL13 directly correlated with disease activity in UC, the GATA3+ FoxP3+ T cells inversely correlated with disease activity in CD. These findings suggest that the inflammatory environment of IBD may promote increased plasticity between multiple functionally distinct T cell populations, which differ between UC and CD.

✉ James D. Lord
jlord@benaroyaresearch.org

[1] Virginia Mason Medical Center, 1100 Ninth Ave., Mailstop C3-GAS, Seattle, WA 98101, USA

[2] Benaroya Research Institute, 1201 Ninth Ave., Mailstop IN-RC, Seattle, WA 98101, USA

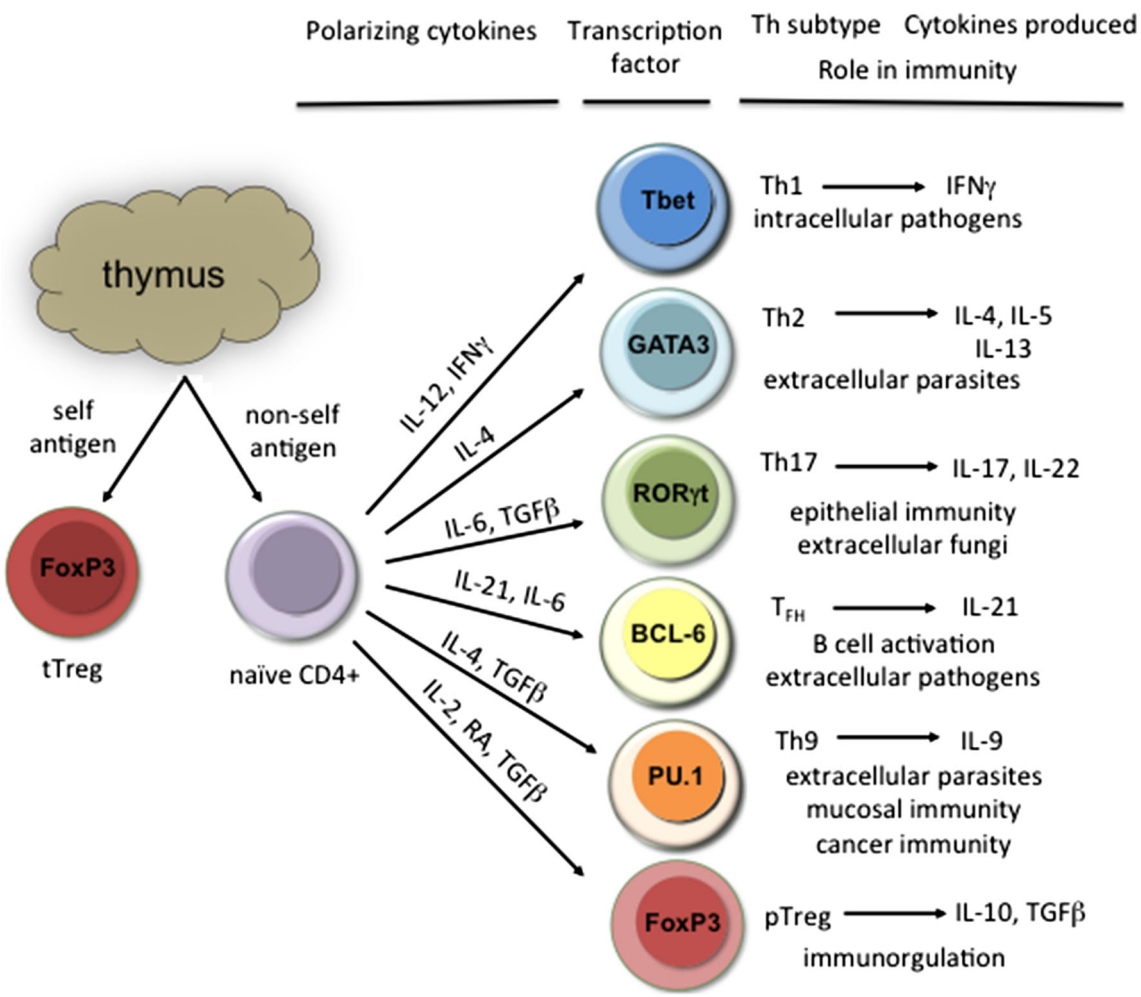


**Fig. 1** CD4+ T cells develop in the thymus. In response to engagement with self-antigens, T cells emerge as FoxP3+ CD4 natural Tregs (nTregs) and in response to engagement with non-self-antigens T cells emerge as naïve T cells. In the periphery, naïve CD4+ T cells encounter polarizing cytokines that result in expression of specific transcription factors that define the specific T helper program. The T helper program is characterized by production of characteristic cytokines. The role of the T helper subtype in immunity is described below the cytokine production profile. tTreg, thymic-derived regulatory T cell; FOXP3, forkhead box P3;GATA3, GATA-binding protein 3; ROR, retinoic acid receptor-related orphan receptor; BCL-6, B cell lymphoma 6; IFNγ, interferon-γ; IL, interleukin; TGFβ, transforming growth factor-β; RA, retinoic acid; TFH, T follicular helper; pTReg cell, peripherally derived regulatory T cell

How might such phenotypic plasticity contribute to IBD pathogenesis? That depends upon the origin of these crossover T cells. The majority of FoxP3+ T cells are generated in the thymus (tTregs) in response to binding of the T cell receptor (TCR) to self-antigens. FoxP3+ Tregs can alternatively be generated from conventional CD4 T cells in the gut-associated lymphoid tissue when they are exposed to retinoic acid and TGFβ-producing dendritic cells. This acquired FOXP3 expression has been associated with regulatory function and impaired pro-inflammatory cytokine expression, as in tTregs. These peripherally generated "pTregs" are more common than tTregs in the intestinal mucosa and likely are vital to limiting inflammation to harmless non-self-antigens from the environment, including food and commensal flora, which tTregs would not have encountered in the thymus. Thus, expression of FOXP3 marks populations of T cells with TCRs specific for antigens against which the immune system should be tolerant.

If FOXP3+ Tregs acquire the ability to express the transcription factors and cytokines typical of pro-inflammatory T cells as a source of the crossover T cells reported by Li et al. [4] and others [5, 6], they would necessarily do so while still maintaining their original antigen specificity. The TCR is a fundamentally immutable aspect of every mature T cell, being encoded in that cell's genome regardless of its phenotypic plasticity. Thus, "crossover" FOXP3+ cells selected to react to self or benign antigens would produce pro-inflammatory factors in addition to, or even instead of, their usual immune-inhibitory functions

when encountering antigens that should normally induce tolerance. Furthermore, if cytokine-secreting FOXP3+ "crossover" cells shared with Tregs, such characteristics as tissue-homing or retention mechanisms, or responsiveness to specific signals in their environment, they would erroneously stimulate inflammation in situations that would otherwise promote tolerance.

Alternatively, FOXP3+ "crossover" cells could be pro-inflammatory T cells that acquire FOXP3 expression as a pTreg, but fail to effectively turn off their existing program. FOXP3 is a transcriptional repressor which under homeostatic conditions blocks T cells from manifesting a pro-inflammatory phenotype. In the case of Th17 cells, the subdomain of FOXP3 encoded by its gene's second exon physically interacts with RORγt, to prevent cells from making IL-17. It is not clear how Treg/Th17 crossover cells continue to make IL-17. Although a splice variant of FOXP3 lacking exon 2 exists in humans, and therefore would not interact with and block RORγt, this is not the sole form of FOXP3 present in IL-17-expressing FOXP3+ T cells from the bowels of people with and without IBD [7]. Instead, another mechanism must be present to impair FOXP3's ability to suppress the pro-inflammatory cytokine production of T cells that express it in IBD.

While it is difficult to determine the origin of a T cell at any given time, a clue as to how it may develop comes from its TCR, as any "sister" cell from which it divided will retain the identical TCR and, if it did not also happen to "crossover," its original phenotype. As TCR diversity is so vast as to make it highly unusual for any two unrelated T cells to accidentally bear identical TCR genes, the TCR profile of phenotypically distinct T cell populations can be sequenced and compared in order to discover overlaps in their respective repertoires which can be presumed to reflect a common ancestry. Intestinal FoxP3+ IL17+ Tregs from CD patients have been previously shown to have TCR Vβ region utilization more similar to Tregs than to Th17 cells [6], suggesting these are Tregs that acquired the ability to secrete IL-17. However, when unique TCR complementarity-determining regions (CDR's) were sequenced, considerable clonal overlap was observed in the colons but not the mesenteric lymph nodes of UC patients between FOXP3+ Tregs (particularly Helios-negative pTregs) and FOXP3-negative effector T cell subsets, reflecting plasticity between mucosal CD4+ T cell populations [8].

The work presented by Li et al. underscores the high degree of T cell plasticity in the inflamed environment of the intestine in inflammatory bowel disease. The majority of the crossover populations described in this study were seen in the LP of IBD populations but to a minimal extent in healthy controls. This implies that environmental cues, likely related to local cytokines, antigenic signal strength and co-stimulatory pathways are significantly altered in the inflamed intestine of the IBD patient. While the percentage of RORγtIL17+ T cells correlated with higher disease activity, GATA3+ FoxP3+ T cells were inversely correlated with disease activity in Crohn's disease. This suggests that inflammation alone is not the sole driver for all types of crossover T cells. Moreover, this may imply a protective function for diversion of FoxP3+ to a Th2 pathway in Crohn's disease, underscoring the importance of T cell plasticity even in disease. A better understanding of the complex cues that allow the redirection of the T cell program from regulatory to inflammatory pathways and vice versa will be indispensable for restoring tolerance in immune-mediated diseases.

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