Plasticity of Th17 and Tregs and its clinical importance as therapeutic target in inflammatory bowel disease

Neeraja Kulkarni¹, Sandip Ashok Sonar², Girdhari Lal³*

¹, ², ³National Centre for Cell Science, Pune, MH-411007, India

Abstract
The gut immune system is very complex and a single layer of the epithelial barrier along the gastrointestinal tract prevents the evasion of various extracellular commensal and pathogenic microorganisms in the gut mucosa. The inflammatory bowel disease (IBD) consists of ulcerative colitis (UC) and Crohn’s disease (CD). The cause of IBD is thought to be associated with genetic, epigenetic, environmental factors, dietary habits and gut microbiota. Immunologically, the effector CD4⁺ T cells such as Th1, Th17, and regulatory CD⁴⁺ T cells play an important role in the pathogenesis of IBD. During gut inflammation and autoimmunity, these cells show phenotypic and functional plasticity, and differentiate into other lineages as well as have mixed-lineage phenotypes such as Th1-Th17, Th1-Treg, and Th17-Treg. The present review discusses the intrinsic and extrinsic factors that affect the cellular and molecular plasticity of Th17 and Treg, their clinical importance, and how plasticity and reprogramming of these cells can be targeted to control the IBD.

Keywords: Autoimmunity, gut inflammation, Th17 cells, Tregs, mucosal tolerance, ulcerative colitis, Crohn’s disease

Introduction
The mammalian gastrointestinal (GI) tract is a highly complex and compartmentalized organ with varied functions.¹ Apart from the role of the GI tract in the digestion and nutrient uptake, gut-associated tissues constitute the highest number (~70%) of innate and adaptive immune cells in the body.²⁻⁴ The two common inflammatory bowel diseases (IBD) are ulcerative colitis (UC) and Crohn’s disease (CD). UC affects only the colon, but CD affects most parts of intestine and colon. The major differences in UC and CD are listed in Table 1. According to a 2010 estimate on IBD burden, India is the second most prevalent country (1.4 million affected cases) after the USA (with 1.64 million cases).¹ In India, the mean age of diagnosis of UC and CD was 38.5 and 35.9 years, respectively.⁵, ⁶ Indian UC patients show a positive family history of about 1.5-2.3%, similar to other Asian countries like Sri Lanka, Japan, South Korea and China.⁵, ⁶

Intestinal epithelial cells form a physical barrier that separates trillions of luminal microflora and the exogenous environment from underlying lamina propria (LP).⁷ Detection of pathogens by host antigen-presenting cells (APCs) leads to cytokine secretion, recruitment, and polarization of CD⁴⁺ T cells. Depending on the cytokines present in the microenvironment, CD⁴⁺ T cells can differentiate into Th1, Th2, and Th17 and Treg cells. Th1 cells express lineage-specific transcription factor T-bet (TBX21) and secret IFN-γ and help in controlling intracellular bacterial and viral infection. Th2 cells express lineage-specific transcription factor GATA3 and produce IL-4 and IL-13, and help in humoral immunity and controlling extracellular parasites.⁸ Th17 cells, marked by transcription factor RORγt, produce IL-17 and IL-21 and help in controlling extracellular pathogens, fungi and promote autoimmunity. Tregs, expressing transcription factor FoxP3, controls the immune response and help in maintaining peripheral tolerance.⁹

Equilibrium between regulatory CD⁴⁺ T cells (Tregs) and effector CD⁴⁺ T cells is critical for balancing gut homeostasis and inflammation. Increasing evidence has proven that among various effector CD⁴⁺ T cells, Th1 and Th17 cells are the most important CD⁴⁺ T cell subset required to confer the protection against microbial pathogens at mucosal surfaces.¹⁰, ¹¹ Th17 cells abundantly exist in the LP of the small intestine¹² and they not only protect the host against infection, but their hyperactivation...
### Table 1: Pathological and molecular differences between ulcerative colitis and Crohn’s disease

| Characteristic features                      | Ulcerative colitis                                                                 | Crohn’s Disease                                                                 |
|---------------------------------------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| **Pathological features and distribution**  | Continuous inflammation of the innermost epithelial layer of colon and the rectum and show superficial ulcers in the mucosa and submucosa.\(^{178}\) | Focal, patchy mucosal inflammation all along the gastroenteric (GI) tract and show deep ulcers and cobblestoned appearance.\(^{179}\) |
| **Histopathologic features**                | No granuloma, abundance of lymphocytic population.\(^{178, 180}\)                  | Shows granuloma and increased polymorphonuclear cells.\(^{179, 180}\)            |
| **Symptoms**                                | Bloody diarrhea, abdominal cramps, anemia, weight loss, fever and fatigue.\(^{180}\) | Diarrhea, abdominal cramps, anemia, weight loss, fever and fatigue,\(^{179, 180}\) |
| **Associated complications**                | Severe bleeding, toxic acute colonic distension, rupture of the bowel and colon cancer.\(^{190}\) | Stenosis, abscess, fistulas and colon cancer.\(^{183}\)                           |
| **Risk factor**                             | Smoking: Primarily affects non-smokers and ex-smokers,\(^{181, 182}\)              | Smoking: Both active and passive smokers are more susceptible.\(^{181}\)          |
| **Dietary habits association**              | Food enriched with saturated fats, polyunsaturated fatty acids, omega-6 fatty acids and meat increases the risk of ulcerative colitis.\(^{185}\) Vegetable rich food reduces the risk of ulcerative colitis.\(^{185, 186}\) | Food enriched with saturated fats, polyunsaturated fatty acids, omega-6 fatty acids and meat increases the risk of Crohn’s disease.\(^{185}\) Dietary fiber and fruit reduces the risk of Crohn’s disease.\(^{185, 186}\) |
| **Genetic associations**                    | Genetic variation in NOD2 and PTPN22 are protective in ulcerative colitis patients.\(^{125}\) | Genetic variation in NOD2 and PTPN22 increases the risk of Crohn’s disease.\(^{125}\) |
| **Biased T cell response in the affected tissue** | Atypical Th2 response (IL-5 producing) and IL-13 producing non-classical NKT cells mediate apoptosis of colonic epithelial cell breaks the epithelial barrier system.\(^{187, 188}\) IL-21-expressing CD4\(^+\)CXCR5\(^+\) Tfh cells are also increased in the biopsy samples of ulcerative colitis.\(^{189}\) | Biased Th1/Th17 response.\(^{187, 190}\) Additionally, IL-21-expressing CD4\(^+\)CXCR5\(^+\) Tfh cells are also increased in the biopsy samples of Crohn’s disease patients.\(^{191}\) |
| **Cytokines involved in pathology**         | Predominantly IL-4, IL-5 and IL-13.\(^{187}\) Recently, IL-9 level was also found increased in ulcerative colitis patients.\(^{192}\) | Predominantly IFN-\(\gamma\), TNF\(\alpha\) and IL-17A.\(^{187, 190}\) |
| **T helper cell-associated response**       | Lamina propria T cells express IL-5, IL-13 and GATA3 with low IL-4.\(^{187, 188}\) IL-22-expressing Th22 cells are significantly reduced in ulcerative colitis patients.\(^{193}\) | Lamina propria T cells express IFN-\(\gamma\), IL-12R\(\beta\)2, STAT4 and T-bet.\(^{187, 194}\) No aberrant reduction of Th22 cells found in Crohn’s disease patients.\(^{193}\) |
| **Innate lymphoid cells**                   | Less abundance of CD56\(^+\)IL17A\(^+\)IL-17F\(^+\) ILCs\(^{190}\) and CD3-CD56\(^+\)NKp46\(^+\) (IFN-\(\gamma\)-secreting) ILCs.\(^{196}\) | Increased abundance of CD56\(^+\)IL17A\(^+\)IL-17F\(^+\) ILCs\(^{190}\) and CD3 CD56\(^+\)NKp46\(^+\) (IFN-\(\gamma\)-secreting) ILCs.\(^{196}\) |
| **Role of intestinal epithelial cells**     | Increase IL-33 (alarmin) and IL-37 production was observed in the intestinal epithelial cells (IECs) of ulcerative colitis patients.\(^{197}\) IL-33-deficient mice or transgenic IL-37 mice protected from experimental colitis.\(^{198, 199}\) | |
also cause autoimmune inflammation in the gut. On the other hand, Tregs are critical in maintaining the peripheral tolerance as well as they function as dedicated suppressor cells of immune systems. The Tregs and Th17 cells are characterized by several molecular markers (Fig. 1). The microbiota in the gut has a very strong influence on the frequency of Th17 and Treg cells. It has been shown that germ-free animals have an unbalanced generation of Th17 cells in the gut. In germ-free mice, feeding of segmented filamentous bacteria (SFB) induces IL-17- and IL-22-producing Th17 cells, whereas the mixture of *Clostridium* species can cause higher differentiation of Tregs in the gut. These studies suggest that gut microbiota helps in shaping the gut immunity. Similarly, *Candida albicans*, a fungal infection, increases the production of IL-12 by dendritic cells, thereby promoting the differentiation of IFN-γ-producing Th1 cells. β-glucan, a fungal cell wall component, promotes Th17 cell differentiation by enhancing the production of prostaglandin E2 (PGE2). Several other dietary components such as vitamin A, vitamin B3 (niacin), vitamin D, tryptophan and food fibers are known to affect the CD4+ T cell differentiation and plasticity. Several extrinsic and intrinsic factors that can affect the development and pathogenesis of IBD, and many of these factors influence

| Immunotherapy attempts | Anti-IL-13 (anruckinzumab and tralokinumab) failed to show efficacy in phase 2 clinical trials of ulcerative colitis patients. Anti-IL-4/13 bi-specific antibody showed better protection in oxazolone-induced murine colitis. | Anti-IFN-γ (fontolizumab) did not show efficacy in phase 2 trial, while anti-TNF-α (infliximab) showed some efficacy. Humanized anti-IL-17A (secukinumab) did not show promise in phase 2 trials rather induced adverse side effects in Crohn’s disease patients. Recombinant human IL-10 therapy was ineffective. SMAD7 antisense oligonucleotide therapy (improves TGF-β-dependent Treg mediated suppression) showed some promise in phase 1 and phase 2 trial. Anti-p40 subunit of IL-12 and IL-23 (briakinumab and ustekinumab) showed better results in Crohn’s disease patients that did not respond to anti-TNF-α treatment. Ustekinumab (September 2016) approved by US FDA for treatment for CD patients who are refractory to conventional and/or anti-TNF therapy. |

![Fig. 1: The phenotype of Th17 and Treg cells](image)

(A) Variety of receptors, transcription factors, cytokines and chemokines associated with the Th17 cell. (B) Variety of receptors, co-stimulatory molecules, regulatory molecules, transcription factors and cytokines associated with the Treg cell.
the developmental and functional plasticity of Th1, Th2, Th17 and Treg cells (Fig. 2). The present review discusses the cellular and molecular mechanisms that influence the plasticity of Treg and Th17 cells and its importance in IBD.

**Differentiation and function of Tregs**

There are several mechanisms by which intestinal immune homeostasis and tolerance are regulated, and among them, FoxP3-expressing CD4$^+$ Tregs play a major role. Depletion of the CD25$^+$CD4$^+$ Tregs leads to the generation of multi-organ autoimmunity including gastrointestinal inflammation. Further, co-transfer of CD4$^+$CD25$^+$ Tregs along with CD4$^+$CD45RB$^{hi}$ naïve T cells into RAG1$^{-/-}$ mice could suppress the chronic intestinal inflammation caused by excessive differentiation of naïve T cells into inflammatory effector cells, indicating the importance of Tregs in controlling the inflammation and autoimmunity. FoxP3 is a crucial transcription factor required for differentiation of bonafide Tregs, and a mutation in the mouse FoxP3 gene leads to a scurfy phenotype, which is a multi-organ inflammatory condition including gut inflammation. Moreover, mutations in the human FoxP3 locus also result in an immunodysregulation, polyendocrinopathy, enteropathy and X-linked (IPEX) syndrome.

**Fig. 2: Plasticity of Treg and Th17 cells in gut inflammation and inflammatory bowel disease**

Naïve CD4 T cells under influence of TGF-β and IL-2 differentiate into the induced Treg (iTreg) and expresses FoxP3 and help in establishing the peripheral tolerance. Under influence of inflammatory signal, iTreg can be differentiated into FoxP3 and RORyt-expressing Treg-Th17 cells. Naïve CD4$^+$ T cells under influence of TGF-β, IL-6 or IL-1β and IL-23 can differentiate into Th17 or Th1-like Th17 cells, respectively. In the presence of high concentration of CCL20, iTreg can also be differentiated into Treg-Th17 cells. Th17 cells can also differentiate into pathogenic Th1-like Th17 cells by CCL20 and IL-23 stimulation in the gut. Th17 cells under the influence of TGF-β can differentiate into the IL-10 producing exTh17/Tr1 cells and that shows regulatory function in the gut. Th1-like Th17 cells under influence of IL-12 rich environment can differentiate into exTh17-Th1 cells and acquire strong pathogenic phenotype.
syndrome. Together, these observations very well establish the key role of FoxP3+ Tregs in maintaining the immune tolerance throughout the body including the gut.

Almost every organ of the human body has FoxP3+ Tregs and account for about 5-10% of total CD4+ T cells that express FoxP3 in the peripheral blood. Interestingly, the intestinal lamina propria (LP) contains much higher numbers of Tregs (~30% of CD4+ T cells in the colonic LP and ~20% in the small intestinal LP). Intestinal Tregs regulate gut immune responses through various molecular mechanisms. Intestinal Tregs constitutively express suppressive molecule CTLA4, co-stimulatory molecule ICOS, and anti-inflammatory cytokines such as TGF-β and IL-10, and these molecules inhibit the immune response against dietary antigens and intestinal microbiota.

When the gut microbiota is absent in the case of germ-free mice or antibiotic-treated mice, the number of colonic Tregs severely impaired, and moreover Tregs cells present in the colon LP show significant low expression of CTLA4, ICOS, and IL-10, indicating impaired regulatory function. Further, when germ-free mice were administered with sterile food, the number of small intestine Treg was significantly compromised, but a number of colonic Tregs were unaffected. These findings suggest that dietary antigens and microflora help in the generation of intestinal Tregs.

There are two types of Tregs. Tregs generated in the thymus are named as natural Tregs (nTregs), and Tregs generated in the periphery called as induced Tregs (iTregs). The intestinal microenvironment favors the generation of iTregs. Adoptive transfer of naive FoxP3+CD4+ T cells isolated from TCR-transgenic mice into wild-type mice, followed by oral administration of cognate antigen, resulted in the accumulation of iTregs. Moreover, transfer of FoxP3+ nTregs alone to FoxP3-deficient mice could not completely overcome the mortality caused by FoxP3 deficiency, underlining the non-redundant immunoregulatory role of iTreg cells. Even though iTregs and nTregs share same phenotype and function, there are distinct differences between them. FoxP3 expression is regulated by three different conserved non-coding sequences (CNS), CNS-1 to CNS-3. Structure of Foxp3 regulatory elements is shown in figure 3. In nTregs, initial induction of FoxP3 expression is mediated by CNS-3, and CNS-3 recruits the transcription factor REL to the FoxP3 locus. The deficiency of CNS-3 leads to significantly impaired nTregs development, but not iTregs. On the other hand, CNS-1 is required for iTreg development, but not CNS-3. CNS-1 acts as a response element for the TGF-β–SMAD signaling pathway and it also contains the binding site for the retinoic acid receptor, which is a heterodimer consisting of retinoic acid receptor (RAR) and retinoid X receptor (RXR). Deficiency of CNS-1

Fig. 3: Structure of murine Foxp3 regulatory elements

Transcription start site (TSS) is marked as +1. Non-coding enhancer elements (CNS1 and CNS 2) are located between -2a and -1 intron. CNS3 is located between exon 1 and exon 2. Activation of proximal promoter, CNS2 and CNS3 help in the generation of iTreg and nTreg. Demethylation of CpG islands present at upstream sites (~5.7kb) and CNS2 region play role in stabilizing the FoxP3 expression. Binding of various transcription factors at specific sites is given in boxes.
disturbs iTreg development in the gut and lungs, but nTreg remains unaffected. Interestingly, CNS-1 deficient mice spontaneously induce Th2-mediated pathology in the gut and the lungs, demonstrating the significance of iTreg cells in the suppression of Th2-mediated immune response at mucosal sites.

Comparisons between in vivo iTregs and in vitro FoxP3-transfected CD4+ T cells indicated significant differences in gene expression patterns. For Treg-lineage commitment and their suppressive function, Treg-specific epigenetic changes are also essential. For stable Treg differentiation and function CNS-2 of the FoxP3 gene locus and various other regulatory elements of Treg-specific genes, such as Cta4 and glucocorticoid-induced TNFR-related (GITR) are also required to be fully demethylated. The demethylation of these Treg-specific gene loci are independent of FoxP3 expression. IL-2 signaling activates STAT5 in CD4+ T cells and activated STAT5 binds to the CNS-2 enhancer of FoxP3 locus. Demethylation of CNS-2 results in a stable expression of FoxP3. With the help of transcription factor Runx1, FoxP3 itself can bind to its own locus at demethylated CNS-2, which further stabilizes the expression of Foxp3. The CNS-2-deficient Tregs readily lose FoxP3 expression in response to proinflammatory cytokines and strong T cell receptor (TCR) stimulation, especially in the intestine, liver, and lungs, suggesting its important role in the stability of Treg.

Even though Tregs maintain their phenotypic stability, they may undergo functional plasticity to respond to the changing microenvironment. Foxp3+ Tregs can express effector T cell-specific transcription factors. Approximately, 65% of the Treg cell population in the colon and around 35% in the small intestines express RORγt. Under germ-free conditions, RORγt+ Tregs disappear, and they do not express neuropilin-1 (NRP1) and Helios, a marker for nTreg indicating that they are iTreg cells generated in response to microbial antigens. A significant decrease RORγt+ Tregs in the mice having a Treg-specific deficiency of STAT3 indicates that STAT3 is required for the generation of RORγt+ Treg lineage. Proinflammatory cytokines such as IL-6 and IL-23 are known to activate STAT3 followed by RORγt, but the precise mechanism of differentiation of RORγt+ Treg is still not very clear. Apart from the RORγt expression, nearly all colonic Tregs show a CD44hi effector Treg-like phenotype and maintenance of this phenotype requires continuous TCR signaling and interferon regulatory factor 4 (IRF4) expression.

LP CD103+ DCs are a potent antigen presenting cells (APCs), and they extend their dendrites through the IEC layer to sample luminal antigen. These DCs can also procure luminal antigens by a distinct transport system through goblet cells. Apart from antigen presentation, CD103+ DCs also express αvβ8 integrin that converts latent TGF-β into its active form and also express enzyme alcohol dehydrogenase (ALDH), which break down vitamin A into retinoic acid. By producing active TGF-β and retinoic acid, CD103+ DCs promote the biased generation of Tregs. Thus, the microenvironmental cues in the LP and mLNs lead to the differentiation, stabilization, proliferation and functional plasticity of Tregs to maintain intestinal immune homeostasis.

**Differentiation and function of Th17 cell**

The IL-17A-producing Th17 cells are ubiquitously present in the GI tract of patients with IBD. The enhance levels of serum and mucosal IL-17A is reported in UC and CD patients. Further, gene polymorphism at IL-17A locus has been shown to link with UC susceptibility, indicating the importance of Th17 cells in the gut susceptibility. IL-17A is a proinflammatory cytokine and induces strong neutrophil infiltration and granulopoiesis in the intestine to clear bacterial and fungal infections along with promoting barrier function of IECs. Even though Th17 cells are specially programmed to respond against mucosal pathogens, they are also known to play the central role in the pathogenicity of several autoimmune diseases including autoimmune colitis. TGF-β1 and IL-6-induced Th17 cells are not competent to induce FoxP3 expression. c-Maf is also known to regulate IL-22 expression. IL-21 teams up with TGF-β to induce expression of RORγt, a master regulator of the Th17 cells followed by IL-17A production, and it also imparts the IL-23R expression on the surface. IL-21 suppresses the differentiation of TGF-β-induced Foxp3+ Tregs in an IL-6-independent manner to further support the Th17 polarization. The IL-6 elicits IL-21 production in Th17 cells in a c-Maf-dependent manner, and IL-21 functions in an autocrine fashion to upregulate IL-23R expression. c-Maf is also known to regulate IL-10 expression during Th17 polarization.
10, 81 IL-23 treatment to TGF-β1 and IL-6 induced Th17 cells result in the enhanced ability of Th17 cells to cause autoimmune. 82 Further, the polarization of murine Th17 can also be mediated in the absence of TGF-β1 and can be induced by the combination of IL-1β, IL-6, and IL-23 or with TGF-β3 and IL-6. 83, 84 Thus, differentiation of Th17 cells is dependent on distinct combinations of cytokines present in the microenvironment. As described earlier RORγt acts as a master regulator of Th17 cells, but it should cooperate with an array of other transcription factors like STAT3, interferon regulatory factor 4 (IRF4), and basic leucine-zipper ATF-like transcription factor (BATF) to switch on the Th17 polarization program.

IL-6 and IL-23 activate STAT3 in the CD4+ T cells and activated STAT3 is indispensable for the differentiation of Th17 cells, as the expression of RORγt and IL-17 is STAT3-dependent. 77 CD4+ T cells-specific deletion of STAT3 leads to hampered in vivo IL-17A production and suppression of IL-17A-mediated pathology. 86-88 STAT3 binds to the promoter as well as enhancer regions of many Th17-associated genes. 89 IL-17A and IL-17F loci possess several STAT3-binding sites not only in the promoter region but also in the CNS located in the intergenic region. 89 Apart from IL-17, STAT3 regulates the expression of IL-21, IL-21R, and IL-23R in CD4+ T cells, and thus help polarization of Th17 lineage. 94 STAT3 also controls the expression of transcription factors like BATF and IRF4, which are necessary for the polarization of Th17 cells. 88

T cell receptor (TCR) signaling in CD4+ T cells induces expression of BATF, a member of the activator protein 1 (AP-1) transcription factor family. 89, 91 BATF is essential for Th17 polarization, as BATF-deficient mice have significantly reduced susceptibility towards Th17-mediated experimental autoimmune encephalomyelitis (EAE). 91 BATF-deficient murine CD4+ T cells possess normal TGF-β and IL-6 signaling and can express RORγt during T cell development, but they fail to sustain RORγt expression. 91 This phenotype of BATF-deficient murine CD4+ T cells can be partially rescued by overexpressing RORγt, indicating that RORγt and BATF both are essential to induce Th17 polarization. 91 Further, BATF forms a dimer with another transcription factor JUN-B and regulates expression of IL-17, IL-21, and IL-22 in Th17 cell. 91

Like BTAF, transcription factor IRF4 is also critical for the Th17 differentiation, which was confirmed by the fact that IRF4-deficient mice are resistant towards EAE and have a defect in the generation of Th17 response due to hampered RORγt expression. 92 Incomplete rescue of IL-17 expression in IRF4−/− CD4+ T cells by over-expression of RORγt indicates the need for both RORγt as well as IRF4 in the establishment of the Th17 phenotype. 92 A recent study showed that the BATF and IRF4 are initial factors that cooperatively bind and make chromatin accessible for RORγt binding. 93, 94 Hypoxia-inducible factor-1α (HIF-1α) is a responder transcription factor of hypoxia and induced by hypoxia conditions associated with tissue inflammation, TCR signaling and IL-6 signaling in a STAT3-dependent manner. 95 HIF-1α induces expression of RORγt and further team up with p300 and RORγt to induce expression of IL-17A. 96 Runx family includes set of transcription factors that are involved in CD4+ T cell development and differentiation. Mammalian Runx family possesses three transcription factors Runx1, Runx2, and Runx3. Runx1 is essential for thymic T cell development and also involved in Th17 differentiation. 97 Runx1 interact with RORγt and bind on the IL-17 promoter to control the Th17 differentiation. 97

The aryl hydrocarbon receptor (AHR) is an evolutionarily conserved transcription factor, and it can bind to a variety of small synthetic compounds and natural ligands. Th17 cells express AHR, and upon ligand binding, AHR undergoes conformational change. 99 Activated AHR migrates to the nucleus and enhances the production of IL-17A, IL-17F, and IL-22. 98, 99 AHR-deficient mice develop less severe EAE indicating the role of AHR in Th17-mediated pathogenicity. 100 TCR signaling enhances intracellular Ca++ that results in the dephosphorylation and activation of nuclear factor of activated T cells (NFAT) transcription factors. Human and murine IL-17 promoter has multiple binding sites for NFAT. 101 The presence of NFAT1 in CD4+ T cells is associated with higher expression of IL-17. 102 Further, factors that can oppose the NFAT binding to DNA can suppress the IL-17 expression.

**Th17 and Treg plasticity and its clinical importance**

Intestinal CD4+ T cells have to exhibit a functional plasticity to deliver a quick response to ever-changing microenvironmental cues. Tregs and Th17 cells are functionally antagonistic to each other, but they display several common features. Treg and Th17 differentiation shares some common signaling mechanisms and key transcriptional factors. These two populations are accumulated abundantly at the mucosal sites like intestine. 103, 105 TGF-β, a pleiotropic cytokine, plays an important role in the differentiation of both the subsets.
Even though TGF-β is indispensable for the generation of Tregs, it is redundant for Th17 differentiation.\textsuperscript{106, 107} Thus, intestinal CD4\(^+\) T cells are a mixture of functionally and phenotypically intermediate subpopulations along with the conventional Tregs and Th17 cells and can interconvert their effector or suppressor function in response to different environmental cues.\textsuperscript{108} The phenotypic plasticity of Tregs and Th17 cells and its role in the gut microenvironment are illustrated in fig. 2.

Various studies have proven that FoxP3\(^+\) Tregs are neither phenotypically nor functionally stable and they can trans-differentiate into Th17-like cells. Treg plasticity was first reported in murine system, where murine Foxp3\(^+\)CD4\(^+\) cells could convert to Th17 cells by IL-6 in the absence of TGF-β,\textsuperscript{109-113} and the observation was further reproduced in humans too.\textsuperscript{109, 114, 115} These Th17-like Tregs produce IL-17 and even though they possess the suppressive capacity \textit{in vitro}, upon treatment with IL-1β and IL-6, the cells lose suppressive capacity.\textsuperscript{115} Under arthritic conditions, CD25\(^+\)FoxP3\(^+\)CD4\(^+\) T cells lose FoxP3 expression and infiltrate into the inflamed joints.\textsuperscript{116} Adoptive transfer of these cells into mice leads to the generation of rapid and severe arthritis.\textsuperscript{116} IL-17-producing FoxP3\(^+\)CD4\(^+\) T cells are also prevalent in IBD patients.\textsuperscript{109, 117} These cells have enhanced responsiveness towards Th17-produced cytokines in IBD patients, and it is yet elusive if they retain their suppressive function.\textsuperscript{117} This context-dependent plasticity adds to the complexity of the functional and phenotypic overlap between Treg and Th17 cells observed in IBD.\textsuperscript{117-119} TGF-β1 signaling plays very important role in the plasticity of Th17, Tregs and Th17-Tr1 cells. TGF-β1 signaling molecule SMAD7, which negatively regulates the activation of SMAD2/3 complex, have been targeted to control IBD. Mongersen (GED 0301), a specific SMAD7 anti-sense oligonucleotide that targets SMAD7 mRNA for degradation was used in the phase 2 clinical trial in CD patients by formulating a way to deliver into the lumen of ileum and colon. The Mongersen showed clinical remission (55% with 40mg and 65% with 160mg) as compared to placebo (10%; \(p<0.001\)).\textsuperscript{120, 121} Currently, the two-randomized multicenter phase 3 clinical trial as induction and maintenance therapy for active CD (Clinical trial registry number NCT02685683 and NCT02641392) as well as an open-label phase 3 trial in CD (NCT02685683) and a multicenter phase 2 trial on UC (NCT02601300) with mongersen are in progress.\textsuperscript{122}

Recently, Aiolos, a member of the Ikaros family, has been found to promote Th17 differentiation by suppressing IL-2 production.\textsuperscript{123} Interestingly, the Helios-deficient iTreg expressed high amounts of Aiolos, and these Helios Aiolos\(^+\) FoxP3\(^+\)CD4\(^+\) T cells express high amount of IL-17 and have hampered suppressive function.\textsuperscript{124} Moreover, polymorphism at Aiolos locus has been shown to associate with CD and UC.\textsuperscript{125} Thus, Aiolos may be an important mediator of Th17 plasticity during intestinal inflammation. Basu \textit{et al.} (2015) showed that SOCS3, an inhibitor of STAT3, is repressed by IL-1β leading to a disturbed STAT3/STAT5 ratio, resulting in Th17 generation.\textsuperscript{126} This can explain the mechanism of trans-differentiation of FoxP3\(^+\) Tregs to FoxP3\(^+\)IL-17\(^+\) CD4\(^+\) T cells by exogenous IL-1β.\textsuperscript{126} Many of the drugs/biologics that target the plasticity of helper CD4 T cells such as tocilizumab (block IL-6R signaling), tofacitinib (an oral small-molecule Janus kinase 3/1 inhibitor) along with drugs that target TNF-α (infliximab, adalimumab and golimumab) and integrin α4β7 (vedolizumab) were investigated as treatment for IBD.\textsuperscript{122, 127} IL-6 signaling plays very important role in Th17 cell differentiation, and IL-6 and soluble IL-6 receptor (sIL-6R) is highly expressed in patients with IBD. Tocilizumab (humanized anti-IL-6R mAb), olokizumab (CDP6038), clazakizumab (BMS945429) and PF-04236921 (human IL-6 mAb) are the antibodies developed against IL-6.\textsuperscript{122} The Janus kinases (JAK1, 2, 3 and TYK2) are upstream to STAT3 signaling and they control the differentiation of Th17 and Treg cells. Several small molecule inhibitors (>1kDa), that can diffuse in the cells are developed to target Janus kinases that may be effective in controlling several autoimmune diseases including IBD. Tofacitinib, an oral small molecule that inhibits mostly JAK1 and JAK3, and prevents signaling from IL-2, IL-4, IL-6, IL-7, IL-9, IL-15, IL-21 and IFN-γ, have a very potent immunosuppressive function.\textsuperscript{128} The phase 3 clinical trial with tofacitinib 10mg twice daily (BID) for 8 weeks in UC patients showed clinical remission (18.5%), clinical response (59.9%), mucosal healing (31.3%) compared to placebo (8.2%, 32.8% and 15.6%, respectively).\textsuperscript{128, 130} The other selective JAK1 inhibitors namely filgotinib (GLPG0634), JNJ-54781532 (ASPK015K) and upadacitinib (ABT-494) are in phase 2 and phase 3 studies for UC and CD.\textsuperscript{128} The filgotinib, an oral JAK1-small molecule inhibitor with better safety profile,\textsuperscript{131} is currently being investigated in two phase 3 trials in CD patients (NCT02914561 and NCT02914600) and two phase 3 trials in UC patients (NCT02914522 and NCT02914535).

There are several studies that showed a subset of Th17 cells that can produce IFN-γ along with IL-17 in the inflamed intestine and these cells are called as Th1-like
These Th1-like Th17 cells can either retain or lose their capacity to produce IL-17A while acquiring expression of IFN-γ. This process is mediated by IL-12 and IL-23 in a STAT4 and T-bet-dependent manner. IL-17:IFN-γ Th17 cells lack colitogenic potential. However, IL-17:IFN-γ Th17 cell can induce experimental colitis. A phase 2 clinical trial involving active CD patients has evaluated laquinimod, an oral small molecule that suppresses the Th1 and Th17 cells and drive the differentiation of Th2 cells. Recently, FOS-like antigen 2 (Fosl2) has been discovered as a key regulator of Th17 plasticity. Culturing of Fosl2-deficient CD4+ T cells under Th17 lineage-specific conditions have induced their conversion to IL-17-producing cells expressing FoxP3. Interestingly, lack of Fosl2 also induced significant production of IFN-γ in Th17 as well as Th2 cultures, specifically when Th17 cells were subsequently exposed to Th1-specific conditions. Genetic, epigenetic, environmental, and dietary factors influence the development of IBD and these factors may also have the direct or indirect effect on function and plasticity of Treg and Th17 cells. Although, Th17 cells have a good contribution to the development of IBD, blocking of IL-17A with mAb secukinumab fails to inhibit IBD. Similarly, IL-17 receptor monoclonal antibody brodalumab fails to give any relief to IBD. The clinical trials of anti-IFN-γ mAb ontolizumab showed no benefits, suggesting that targeting Th17 and Th1 cells alone is not very effective in IBD. It has been shown that UC patients had an increased frequency of CCR6+RORγt+ CD4+ T cells in the peripheral blood as compared to the healthy individuals. IBD patients had a higher frequency of FoxP3+IL-17+ and IL-17+IFN-γ+ CD4+ T cells as compared to healthy individuals. The IL23 receptor-expresses on pathogenic Th17 cells and is composed of two subunits IL-12 receptor-beta1 chain (IL12Rβ1) and IL-23 alpha subunit (IL23A). The IL-12Rβ1 subunit is also expressed on Th1 cells. Currently, several of antibodies that target IL-12 and IL-23 such as ustekinumab (fully human anti-p40 mAb), AMG139/MEDI20170 (fully human anti-p19 mAb), BI-655066 (fully human anti-p19 mAb) and LY3074828 (humanized anti-p19 mAb) are being investigated in phase 2 and phase 3 clinical trials in UC and CD patients. Targeting p40 subunit (shared by IL-12 and IL-23 cytokines) with mAb ustekinumab, an FDA approved biologic, has been demonstrated to have an inhibitory role in IBD patients. Risankizumab, a humanized monoclonal antibody against p19 subunit of IL-23, has a selective advantage of blocking only IL-23, but not IL-12 molecule. A multicenter phase 2 clinical trial with risankizumab in CD with 600 mg mAb showed clinical remission of 42% compared to the placebo 20.9% (p=0.025) with acceptable safety profile. Likewise, any drug, biologics or small molecule inhibitors that target the plasticity of Th17 and Treg may shed a new light on controlling the IBD.

The unique inflamed microenvironment in the gut and associated lymphoid tissues of IBD patients stimulate the generation of selective CD4+ T cell subsets and also tune the plasticity of these cells. Various triggers such as CD103+ dendritic cells (DCs) in response to commensal antigens can be programmed to produce retinoic acid, TGF-β and IL-10, and promote the differentiation of naïve CD4 T cells into FoxP3-expressing iTregs. Similarly, short chain fatty acids (SCFAs) such as butyrate and propionate, produced by gut microbiota during the digestion of fiber-rich food, vitamin B3 and D3, low-affinity aryl hydrocarbon receptor (Ahr) ligand (2,3,7,8-tetrachlorodibenzo-p-dioxin), and these molecules are known to drive the differentiation of iTreg differentiation. The folic acids-derived signals, delivered to the folate receptor 4-expressing nTregs, support their survival in the intestine. Moreover, most of the colonic Tregs exhibit plasticity and co-express FoxP3 and RORγt. The IL-17A in iTregs has been reported to control bacterial infections in the gut. During IBD, the lack of dietary fibers-derived SCFAs and dysbiosis can affect the iTreg differentiation and promote the differentiation of Th17 development in the gut. Conversely, segmented filamentous bacteria support Th17 differentiation via a circuitry mechanism involving intestinal epithelial cells and CD103+DCs. The IL-23 produced by these DCs promotes Th17 cell differentiation. The fungal infection (Candida albicans) or glucan-derived from other fungi can support the generation of Th17 cells (Fig. 2).

Additionally, a high fat diet comprising of polyunsaturated fatty acids favors Th17 than iTreg differentiation. Both Tregs and Th17 cells in the gut express CCR6 and its ligand CCL20 is induced in the inflamed gut. Interestingly, we have shown that CCR6-CCL20 interaction drives the expression of RORγt and IL-17A into iTregs via the Akt-mTOR-STAT3 pathway, suggesting that not only cytokine signaling but also chemokine receptor stimulation fine-tunes the CD4+ T cell phenotype. The iTregs and/or Th17 cells can acquire intermediate ‘Treg-Th17’ phenotype x’ and exhibit reduced suppressive function. The conventional Th17 cells are thought to be protective in nature during homeostasis, nevertheless, they acquire Th1-like phenotype under the influence of IL-23 and IL-12. These Th17 cells either continue to co-express IL-17A and IFN-γ are known as Th1-like Th17 cells or may sometime...
lose IL-17A expression and continue to express only IFN-γ is called exTh17-Th1 cells. IL-6, produced by the microbial stimulation or toll-like receptor 2 (TLR2) activation on APC, can inhibit the differentiation of Tregs in the STAT3 or IRF1-dependent manner, respectively. Both Th1-like Th17 and exTh17-Th1 cells are highly pathogenic in IBD. The blocking of IL-17A has completely failed to protect from IBD, which could be due to the acquisition of Th1-like Th17 cells. The Th17 cells are also reported to lose RORγt and acquire c-Maf under the influence of TGF-β stimulation and produce IL-10 known as exTh17-Tr1 cells (Fig. 2). Such cells are reported to develop in the gut during homeostasis, however, their development and function during IBD are largely unknown.

The chemokine receptors help in the recruitment of immune cells under the homeostatic and inflammatory conditions in the gut. Some of these chemokine receptors such as CCR2, CCR4, CCR5, CCR6, CCR7, CCR8, CCR10, CXCR4, CXCR5, and CXCR6 are expressed by the Th17 and Treg cells. Both in mice models and humans, Th17 cells have shown a very stable expression of CCR6 on most of the Th17 cell subsets. The deficiency of CCR6 or blockade of CCR6 with anti-CCR6 antibody is known to suppress the gut inflammation and autoimmunity. Newer biologics have been developed against CCR6 and its ligand CCL20 to control several autoimmune diseases. Spingosine 1 phosphate (S1P) is known to regulate the migration of cells from egress of cells in peripheral circulation and signaling from S1P receptor signaling also known to control the differentiation and function of Treg cells, endothelial permeability, angiogenesis and apoptosis. Ozanimod, a novel selective S1P receptor modulator, was tested in phase 2 trial as induction therapy in moderate to severe UC patients. The other oral formulation of small molecules that target S1P1 such as etrasimod and amiselimod are in the phase 2 clinical trial.

The gut microbiota has a strong influence on IBD. A fecal microbiota transplantation (FMT), where an infusion of suspension of stool from a healthy person to the GI tract of IBD patients to cure the disease was tried and that are also known to influence the plasticity of Th17 and Treg cells. In the IBD patients, FMT showed clinical benefits but was donor-dependent as well as recipient-dependent, and the effect was selective and transient. A multicenter trial involving 85 patients used an aggressive strategy of FMT via enema for 5 days a week for 8 weeks and achieved 27% steroid-free clinical remission of IBD with FMT as compared to 8% with placebo.

There are several microRNAs known to control the plasticity of CD4 T cells. Recently, some specific microRNA such as miR-598 and miR-642 in the plasma was able to differentiate the CD from the UC. Some of the microRNAs, which are known to influence the differentiation of Th17 and Treg cells, are found to be associated with IBD. Identifying the microRNA that controls the pathogenicity and plasticity of Th17 cells and its association with IBD may assist in diagnosis of IBD and its classification.

Conclusion
There are several causative agents that may contribute to the development of IBD, and to understand the contribution of each component such as genomics (~24,000 genes), transcriptomics (~10^5 RNA transcripts), proteomics (~10^6 proteins), metabolomics (~10^4 metabolites), exposomics (~10^8 compounds) and metagenomics (10^14 microorganisms) require a Systems Biology approach. The microbiota in the gut is very complex and have set of microbiorganism that forms a network of food chain where products generated by one set of the microbiorganism to be used by another set of microorganisms in the same microenvironment. The by-product or metabolites generated by each one of these sets of complex groups of organisms and its effects on the immune system are not clearly understood. Having a systems biology approach and complex bioinformatics tools to map the role of multivariable parameters in IBD may give a better understanding of the IBD pathogenesis and management. Thus, dynamic changes in the intestinal inflammatory milieu lead to the plasticity in the intestinal Tregs and Th17 cells. Uncovering the complexity residing with the plasticity of Tregs and Th17 cells will offer a better understanding of CD4+ T cells mediated pathogenesis of IBD. A better insight of epigenetic plasticity and reprogramming and factors that can oscillate these changes in the effector and regulatory CD4+ T cells may open a new door to design better strategies to control gut-inflammation and autoimmunity.

Abbreviations
APCs, antigen presenting cells; CD, Crohn’s disease; CNS, Conserved non-coding sequences; FMT, Fecal microbiota transplantation; GALT, gut-associated lymphoid tissue; GI, gastrointestinal tract; IBD, inflammatory bowel disease; IECs, intestinal epithelial cells; LP, lamina propria; mAb,
monoclonal antibody; mLN, mesenteric lymph nodes; PGE\textsubscript{2}, prostaglandin E\textsubscript{2}; PP, Peyer’s patch; RA, retinoic acid; SDF-1, stromal cell-derived factor-1; TCR, T cell receptor; UC, ulcerative colitis.

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Competing interests
The authors declare that they have no competing interests.

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*Correspondence: Dr. Girdhari Lal glal@nccs.res.in

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