IJIR. 2017;(1)1:R2 DOI: 10.15305/ijir/v1i1/258

REVIEWS

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

Neeraja Kulkarni[1], Sandip Ashok Sonar[2], Girdhari Lal[3]*

[1, 2, 3]National 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 CD^+ 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).[5] 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 intestine[12] and they not only protect the host against infection, but their hyperactivation

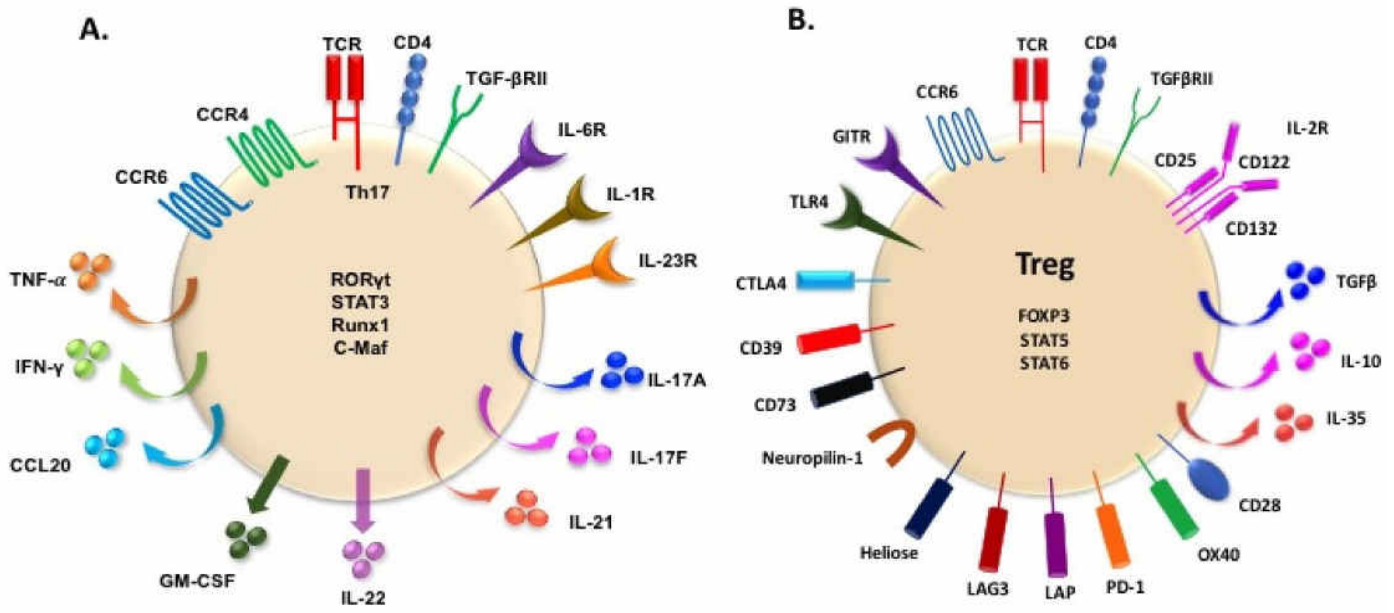
Table 1: Pathological and molecular differences between ulcerative colitis and Crohn's disease

Characteristic features	Ulcerative colitis	Crohn's Disease
Pathological features and distribution	Continuous inflammation of the inner most 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.[180]	Stenosis, abscess, fistulas andmcolon cancer.[180]
Risk factor	Smoking: Primarily affects non-smokers and ex-smokers.[181, 182]	Smoking: Both active and passive smokers are more susceptible.[181]
	Appendectomy: Removal of an inflamed appendix in early life is associated with a decreased incidence, milder disease, and delayed onset of ulcerative colitis.[183]	Appendectomy: Removal of an inflamed appendix in early life is associated with an increased incidence of Crohn's disease.[184]
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 the ulcerative colitis patients.[192]	Predominantly IFN-γ, TNFα 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-γ, IL-12Rβ2, 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 $CD56^-IL17A^+$İL-$17F^+$ ILCs[195] and CD3-$CD56^+NKp46^+$ (IFN-γ-secreting) ILCs.[196]	Increased abundance of $CD56^-IL17A^+$IL-$17F^+$ ILCs[195] and $CD3^-CD56^+NKp46^+$ (IFN-γ-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]	

Immunotherapy attempts	Anti-IL-13 (anrukinzumab and tralokinumab) failed to show efficacy in phase 2 clinical trials of ulcerative colitis patients.[200] Anti-IL-4/13 bi-specific antibody showed better protection in oxazolone-induced murine colitis.[201]	Anti-IFN-γ (fontolizumab) did not show efficacy in phase 2 trial,[202] while anti-TNF-α (infliximab) showed some efficacy.[203] Humanized anti-IL-17A (secukinumab) did not show promise in phase 2 trials rather induced adverse side effects in Crohn's disease patients.[137] Recombinant human IL-10 therapy was ineffective.[204] SMAD7 antisense oligonucleotide therapy (improves TGF-β-dependent Treg mediated suppression) showed some promise in phase 1 and phase 2 trial.[205] 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.[206] Ustekinnumab (September 2016) approved by US FDA for treatment for CD patients who are refractory to conventional and/or anti-TNF therapy.

also cause autoimmune inflammation in the gut.[13] 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.[14-16] In germ-free mice, feeding of segmented filamentous bacteria (SFB) induces IL-17- and IL-22-producing Th17 cells,[17] whereas the mixture of *Clostridium* species can cause higher differentiation of Tregs in the gut.[18, 19] 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.[20] β-glucan, a fungal cell wall component, promotes Th17 cell differentiation by enhancing the production of prostaglandin E2 (PGE2).[21] 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.[22] Several extrinsic and intrinsic factors that can affect the development and pathogenesis of IBD, and many of these factors influence

Fig. 1: The phenotype of Th17 and Treg cells



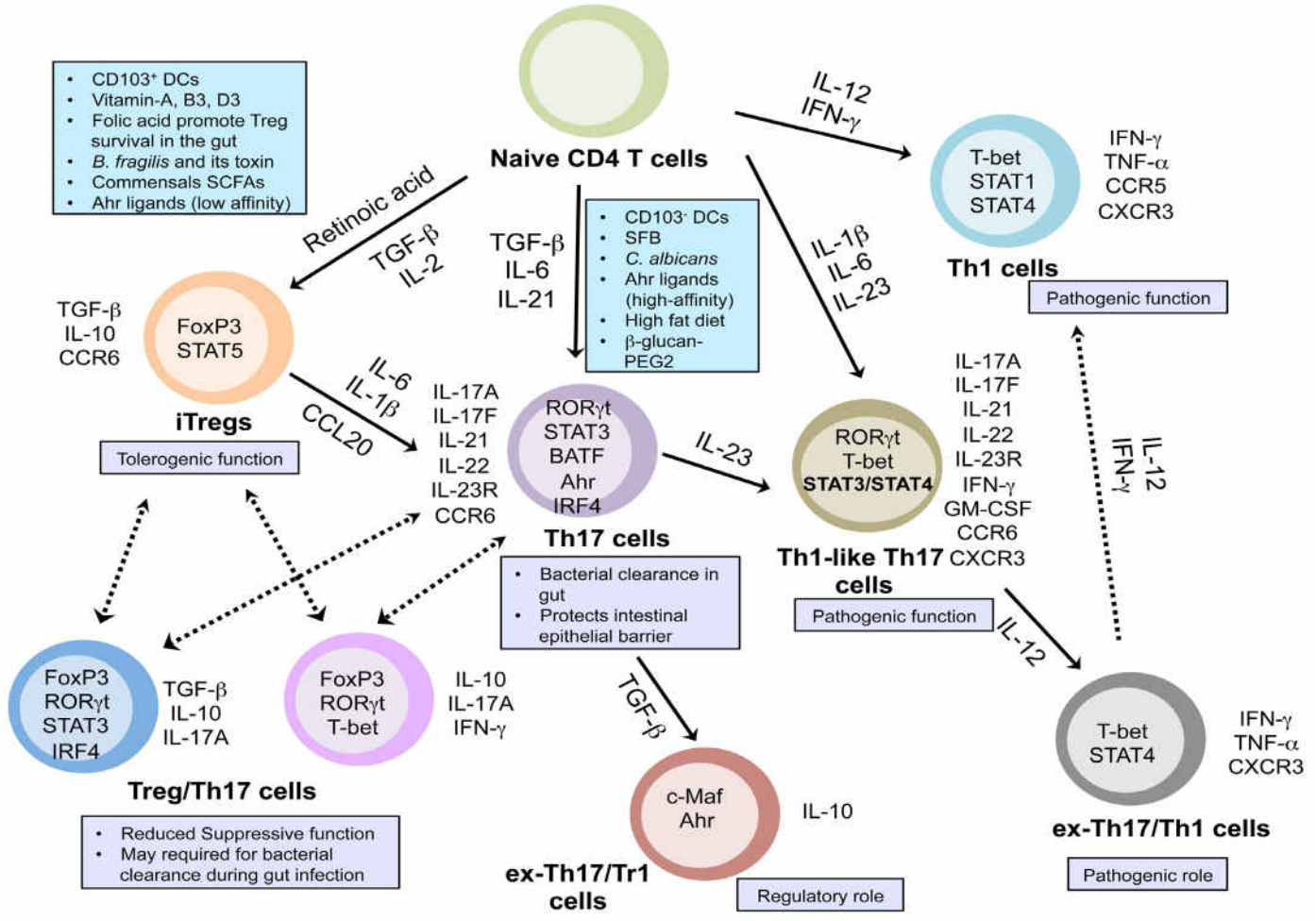
(A) Variety of receptors, transcription factors, cytokines and chemokines associated with the Th17 cell. (B) Variety of receptors, co-stimulatory molecules, regulatory molecules, transcription factors and cytokines associated with the Treg cell

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.[23] 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,[24-26] and a mutation in the mouse *FoxP3 gene* leads to a scurfy phenotype, which is a multi-organ inflammatory condition including gut inflammation.[27] Moreover, mutations in the human *FoxP3 locus* also result in an immunodysregulation, polyendocrinopathy, enteropathy and X-linked (IPEX)

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



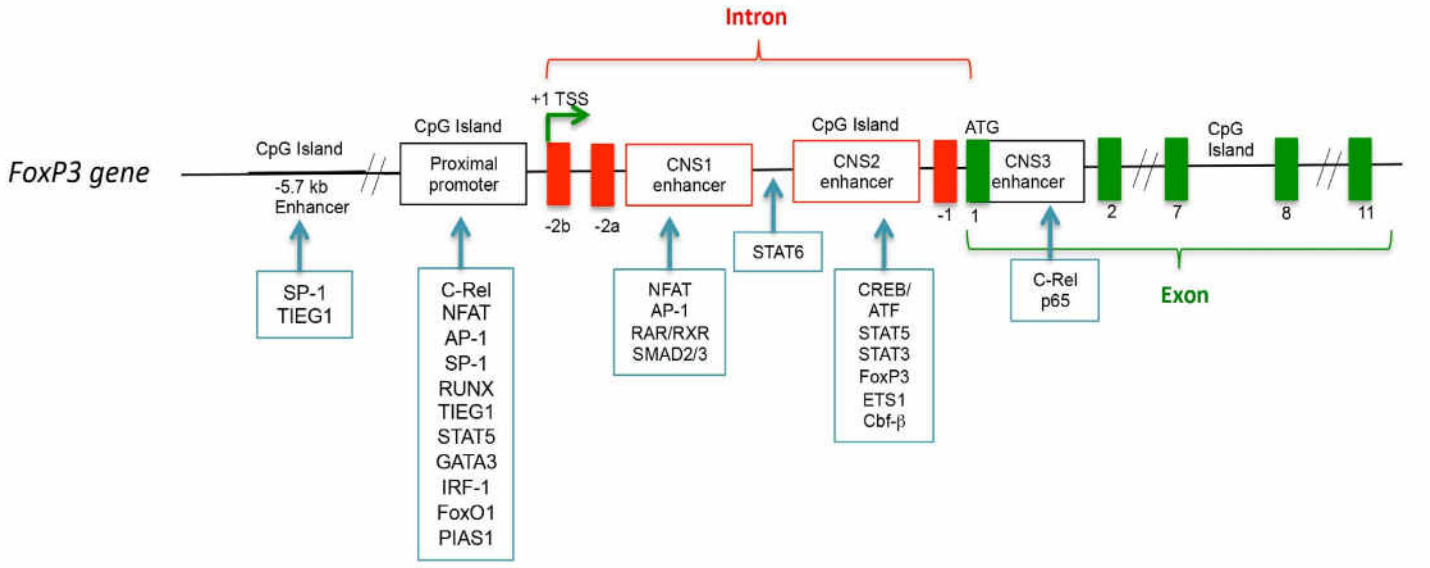
Naïve CD4 T cells under influence of TGF-β and IL-2 differentiate into the induced Treg (iTreg) and expresses FoxP3 and help in establishing the peripheral tolerance. Under influence of inflammatory signal, iTreg can be differentiated into FoxP3 and RORγt-expressing Treg-Th17 cells. Naïve $CD4^+$ T cells 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.[28-30] 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).[31-34] 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.[35-38] 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.[18, 31-33, 39] 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.[39] 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.[40, 41] 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.[42] Even though iTregs and nTregs share same phenotype and function, there are distinct differences between them.[43-46] FoxP3 expression is regulated by three different conserved non-coding sequences (CNS), CNS-1 to CNS-3. Structure of *Foxp3 gene* 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.[47, 48] On the other hand, CNS-1 is required for iTreg development, but not CNS-3.[49, 50] CNS-1 acts as a response element for the TGF-β–SMAD signaling pathway[51] 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).[52] 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.[53] Interestingly, CNS-1 deficient mice spontaneously induce Th2-mediated pathology in the gut and the lungs,[53] 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.[54] For Treg-lineage commitment and their suppressive function, Treg-specific epigenetic changes are also essential.[26, 55] For stable Treg differentiation and function CNS-2 of the *FoxP3* gene locus and various other regulatory elements of Treg-specific genes, such as *Ctla4* 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.[56] 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.[57] 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.[56-58] 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.[49, 57]

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.[14, 15, 59] Under germ-free conditions, $ROR\gamma t^+$ 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.[14, 15, 59] A significant decrease $ROR\gamma t^+$ Tregs in the mice having a Treg-specific deficiency of STAT3 indicates that STAT3 is required for the generation of $ROR\gamma t^+$ Treg lineage.[15] Proinflammatory cytokines such as IL-6 and IL-23 are known to activate STAT3 followed by RORγt[60], but the precise mechanism of differentiation of $ROR\gamma t^+$ Treg is still not very clear. Apart from the RORγt expression, nearly all colonic Tregs show a $CD44^{hi}$ effector Treg-like phenotype and maintenance of this phenotype requires continuous TCR signaling and interferon regulatory factor 4 (IRF4) expression.[61]

LP $CD103^+$ DCs are a potent antigen presenting cells (APCs), and they extend their dendrites through the IEC layer to sample luminal antigen.[62] These DCs can also procure luminal antigens by a distinct transport system through goblet cells.[63] 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.[41, 64] By producing active TGF-β and retinoic acid, $CD103^+$ DCs promote the biased generation of Tregs.[41, 64] 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.[65] The enhance levels of serum and mucosal IL-17A is reported in UC and CD patients.[66] Further, gene polymorphism at IL-17A locus has been shown to link with UC susceptibility,[67] indicating the importance of Th17 cells in the gut inflammation. 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.[68] 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.[69-71] TGF-β1 along with IL-6 efficiently induces Th17 differentiation *in vitro*.[72, 73] Multiple studies have shown that Th17 differentiation can also be promoted by IL-21.[74-76] 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.[76, 77] IL-21 suppresses the differentiation of TGF-β-induced $FoxP3^+$ Tregs in an IL-6-independent manner to further support the Th17 polarization.[74] The IL-6 elicits IL-21 production in Th17 cells in a c-Maf-dependent manner,[78] 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.[79] IL-23-IL-23R interaction stabilizes Th17 lineage by maintaining the expression of RORγt and also enhances the pathogenicity of Th17 cells by inducing the expression of IL-22, GM-CSF, and IL-23R in a Blimp-1-dependent mechanism.[80] Notably, TGF-β1 and IL-6-induced Th17 cells are not competent enough to induce autoimmunity when adoptively transferred *in vivo*, as they co-produce suppressive cytokine IL-

10.[81] IL-23 treatment to TGF-β1 and IL-6 induced Th17 cells result in the enhanced ability of Th17 cells to cause autoimmunity.[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,[85] 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.[88] *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.[84] 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.[90, 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.[95] 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[96] 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[98], 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.[99-101] 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.[102, 103] The presence of NFAT1 in $CD4^+$ T cells is associated with higher expression of IL-17.[104] 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.[77, 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.[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.[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,[109-113] and the observation was further reproduced in humans too.[109, 114, 115] These Th17-like Tregs produce IL-17 and even though they possess the suppressive capacity *in vitro*, upon treatment with IL-1β and IL-6, the cells lose suppressive capacity.[115] Under arthritic conditions, $CD25^{lo}FoxP3^+CD4^+$ T cells lose FoxP3 expression and infiltrate into the inflamed joints.[116] Adoptive transfer of these cells into mice leads to the generation of rapid and severe arthritis.[116] IL-17-producing $FoxP3^+CD4^+$ T cells are also prevalent in IBD patients.[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.[117] This context-dependent plasticity adds to the complexity of the functional and phenotypic overlap between Treg and Th17 cells observed in IBD.[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%; $p<0.001$).[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.[122]

Recently, Aiolos, a member of the Ikaros family, has been found to promote Th17 differentiation by suppressing IL-2 production.[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.[124] Moreover, polymorphism at *Aiolos* locus has been shown to associate with CD and UC.[125] Thus, Aiolos may be an important mediator of Treg-Th17 plasticity during intestinal inflammation. Basu *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.[126] This can explain the mechanism of trans-differentiation of $FoxP3^+$ Tregs to $FoxP3^+IL\text{-}17^+$ $CD4^+$ T cells by exogenous IL-1β.[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.[22, 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. Toclizumab (humanized anti-IL-6R mAb), olokizumab (CDP6038), clazakizumab (BMS945429) and PF-04236921 (human IL-6 mAb) are the antibodies developed against IL-6.[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.[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).[129, 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.[128] The filgotinib, an oral JAK1-small molecule inhibitor with better safety profile,[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

Th17 cells.[132, 133] These Th1-like Th17 cells can either retain or lose their capacity to produce IL-17A while acquiring expression of IFN-γ.[134] 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.[135] A phase 2 clinical trial involving active CD patients has evaluated laquinimod, an oral small molecule that suppress the Th1 and Th17 cells and drive the differentiation of Th2 cells.[136] Recently, FOS-like antigen 2 (Fosl2) has been discovered as a key regulator of Th17 plasticity.[94] Culturing of Fosl2-deficient CD4^{+} T cells under Th17 lineage-specific conditions have induced their conversion to IL-17-producing cells expressing FoxP3.[94] 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.[94] 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.[137] Similarly, IL-17 receptor monoclonal antibody brodalumab fails to give any relief to IBD.[138] The clinical trials of anti-IFN-γ mAb fontlizumab showed no benefits, suggesting that targeting Th17 and Th1 cells alone is not very effective in IBD.[139] 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.[109, 140] IBD patients had a higher frequency of FoxP3^{+}IL-17^{+} and IL-17^{+}IFN-γ^{+} CD4^{+} T cells as compared to healthy individuals.[141, 142] 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).[143-145] 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.[122] 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.[146] 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.[128, 147] 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.[128, 148] 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.[109] 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.[113] 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.[149-152] Both in mice models and humans, Th17 cells have shown a very stable expression of CCR6 on most of the Th17 cell subsets.[153-155] We showed that signaling from CCR6 promotes the pathogenicity of Th17 cells and also controls the plasticity of Tregs using Akt/mTOR/STAT3 pathways in ulcerative colitis and gut inflammation.[109, 156, 157] The deficiency of CCR6 or blockade of CCR6 with anti-CCR6 antibody is known to suppress the gut inflammation and autoimmunity.[109, 153, 158] Newer biologics have been developed against CCR6 and its ligand CCL20 to control several autoimmune diseases.[159-161] Sphingosine 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.[162-165] Ozanimod, a novel selective S1P receptor modulator, was tested in phase 2 trial as induction therapy in moderate to severe UC patients.[166] The other oral formulation of small molecules that target S1P1 such as etrasimod and amiselimod are in the phase 2 clinical trial.[122]

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 were 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.[167-170] 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.[171]

There are several microRNAs known to control the plasticity of CD4 T cells.[110] Recently, some specific microRNA such as miR-598 and miR-642 in the plasma was able to differentiate the CD from the UC.[172] Some of the microRNAs, which are known to influence the differentiation of Th17 and Treg cells, are found to be associated with IBD.[173-176] 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 microorganisms that forms a network of food chain where products generated by one set of the microorganisms 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 a complex group of organisms and its effect on the immune system are not clearly understood. Having a systems biology approach and complex bioinformatics tools to map the role of multi-variable 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_2, prostaglandin E2; PP, Peyer's patch; RA, retinoic acid; SDF-1, stromal cell-derived factor-1; TCR, T cell receptor; UC, ulcerative colitis.

Acknowledgement

This work was supported by Department of Biotechnology (DBT), Government of India (Grants, BT/PR15533/MED/30/1616/2015; BT/PR14156/BRB/10/1515/2016 and BT/PR4610/MED/30/720/2012 to GL). Neeraja Kulkarni and Sandip Ashok Sonar received Senior Research Fellowship (SRF) from Council of Scientific and Industrial Research (CSIR), Government of India.

Author contributions

Neeraja Kulkarni and Sandip Ashok Sonar have equally contributed for publishing the paper.

Competing interests

The authors declare that they have no competing interests.

Citation

Kulkarni N, Sonar SA Lal G. Plasticity of Th17 and Tregs and its clinical importance as therapeutic target in inflammatory bowel disease IJIR. 2017;1(1):R2.

Submitted: 3 January 2018, **Accepted:** 27 February 2018, **Published:** 23 March 2018

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

References

1. Kulkarni N, Pathak M, Lal G. Role of chemokine receptors and intestinal epithelial cells in the mucosal inflammation and tolerance. J Leukoc Biol 2017; 101(2): 377-394.
2. Capitan-Canadas F, Ocon B, Aranda CJ, Anzola A, Suarez MD, Zarzuelo A et al. Fructooligosaccharides exert intestinal anti-inflammatory activity in the CD4+ CD62L+ T cell transfer model of colitis in C57BL/6J mice. Eur J Nutr 2016; 55(4): 1445-54.
3. Eri R, McGuckin MA, Wadley R. T cell transfer model of colitis: a great tool to assess the contribution of T cells in chronic intestinal inflammation. Methods Mol Biol 2012; 844: 261-75.
4. Yoshie O, Imai T, Nomiyama H. Novel lymphocyte-specific CC chemokines and their receptors. Journal of leukocyte biology 1997; 62(5): 634-44.
5. Kedia S, Ahuja V. Epidemiology of Inflammatory Bowel Disease in India: The Great Shift East. Inflammatory Intestinal Diseases 2017; 2(2): 102-115.
6. Makharia GK, Ramakrishna BS, Abraham P, Choudhuri G, Misra SP, Ahuja V et al. Survey of inflammatory bowel diseases in India. Indian J Gastroenterol 2012; 31(6): 299-306.
7. Nelson RT, Boyd J, Gladue RP, Paradis T, Thomas R, Cunningham AC et al. Genomic organization of the CC chemokine mip-3alpha/CCL20/larc/exodus/SCYA20, showing gene structure, splice variants, and chromosome localization. Genomics 2001; 73(1): 28-37.
8. Kanhere A, Hertweck A, Bhatia U, Gokmen MR, Perucha E, Jackson I et al. T-bet and GATA3 orchestrate Th1 and Th2 differentiation through lineage-specific targeting of distal regulatory elements. Nature communications 2012; 3: 1268.
9. Lal G, Bromberg JS. Epigenetic mechanisms of regulation of Foxp3 expression. Blood 2009; 114(18): 3727-35.
10. Khader SA, Gaffen SL, Kolls JK. Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. Mucosal immunology 2009; 2(5): 403-11.
11. Curtis MM, Way SS. Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens. Immunology 2009; 126(2): 177-85.
12. Ivanov, II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 2006; 126(6): 1121-33.
13. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005; 201(2): 233-40.
14. Lochner M, Berard M, Sawa S, Hauer S, Gaboriau-Routhiau V, Fernandez TD et al. Restricted microbiota and absence of cognate TCR antigen leads to an unbalanced generation of Th17 cells. J Immunol 2011; 186(3): 1531-7.
15. Ohnmacht C, Park JH, Cording S, Wing JB, Atarashi K, Obata Y et al. MUCOSAL IMMUNOLOGY. The microbiota regulates type 2 immunity through RORgammat(+) T cells. Science 2015; 349(6251): 989-93.
16. Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D, McGuire AM et al. MUCOSAL IMMUNOLOGY. Individual intestinal symbionts induce a distinct population of RORgamma(+) regulatory T cells. Science 2015; 349(6251): 993-7.
17. Ivanov, II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 2009; 139(3): 485-98.
18. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science 2011; 331(6015): 337-41.
19. Narushima S, Sugiura Y, Oshima K, Atarashi K, Hattori M, Suematsu M et al. Characterization of the 17 strains of regulatory T cell-inducing human-derived Clostridia. Gut Microbes 2014; 5(3): 333-9.
20. Zielinski CE, Mele F, Aschenbrenner D, Jarrossay D, Ronchi F, Gattorno M et al. Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta. Nature 2012; 484(7395): 514-8.
21. Gagliardi MC, Teloni R, Mariotti S, Bromuro C, Chiani P, Romagnoli G et al. Endogenous PGE2 promotes the induction of human Th17 responses by fungal ss-glucan. J Leukoc Biol 2010; 88(5): 947-54.
22. Ueno A, Jeffery L, Kobayashi T, Hibi T, Ghosh S, Jijon H. Th17 plasticity and its relevance to inflammatory bowel disease. Journal of autoimmunity 2017; DOI: 10.1016/j.jaut.2017.12.004.
23. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995; 155(3): 1151-64.
24. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 2003; 4(4): 330-6.
25. Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat Immunol 2003; 4(4): 337-42.
26. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003; 299(5609): 1057-61.
27. Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA et al. Disruption of a new forkhead/winged-helix protein, scurfin,

results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet 2001; 27(1): 68-73.
28. Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C et al. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. The Journal of clinical investigation 2000; 106(12): R75-81.
29. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 2001; 27(1): 20-1.
30. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet 2001; 27(1): 18-20.
31. Weiss JM, Bilate AM, Gobert M, Ding Y, Curotto de Lafaille MA, Parkhurst CN et al. Neuropilin 1 is expressed on thymus-derived natural regulatory T cells, but not mucosa-generated induced Foxp3+ T reg cells. The Journal of experimental medicine 2012; 209(10): 1723-42.
32. Geuking MB, Cahenzli J, Lawson MA, Ng DC, Slack E, Hapfelmeier S et al. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. Immunity 2011; 34(5): 794-806.
33. Stefka AT, Feehley T, Tripathi P, Qiu J, McCoy K, Mazmanian SK et al. Commensal bacteria protect against food allergen sensitization. Proceedings of the National Academy of Sciences of the United States of America 2014; 111(36): 13145-50.
34. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proceedings of the National Academy of Sciences of the United States of America 2010; 107(27): 12204-9.
35. Li MO, Wan YY, Flavell RA. T Cell-Produced Transforming Growth Factor-beta1 Controls T Cell Tolerance and Regulates Th1- and Th17-Cell Differentiation. Immunity 2007; 26(5): 579-91.
36. Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity 2008; 28(4): 546-58.
37. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science 2008; 322(5899): 271-5.
38. Herman AE, Freeman GJ, Mathis D, Benoist C. CD4+CD25+ T regulatory cells dependent on ICOS promote regulation of effector cells in the prediabetic lesion. J Exp Med 2004; 199(11): 1479-89.
39. Kim KS, Hong SW, Han D, Yi J, Jung J, Yang BG et al. Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. Science 2016; 351(6275): 858-63.
40. Curotto de Lafaille MA, Kutchukhidze N, Shen S, Ding Y, Yee H, Lafaille JJ. Adaptive Foxp3+ regulatory T cell-dependent and -independent control of allergic inflammation. Immunity 2008; 29(1): 114-26.
41. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med 2007; 204(8): 1757-64.
42. Haribhai D, Williams JB, Jia S, Nickerson D, Schmitt EG, Edwards B et al. A requisite role for induced regulatory T cells in tolerance based on expanding antigen receptor diversity. Immunity 2011; 35(1): 109-22.
43. Lal G, Zhang N, van der Touw W, Ding Y, Ju W, Bottinger EP et al. Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. J Immunol 2009; 182(1): 259-73.
44. Baron U, Floess S, Wieczorek G, Baumann K, Grutzkau A, Dong J et al. DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3(+) conventional T cells. European journal of immunology 2007; 37(9): 2378-89.
45. Ohkura N, Kitagawa Y, Sakaguchi S. Development and maintenance of regulatory T cells. Immunity 2013; 38(3): 414-23.
46. Hill JA, Feuerer M, Tash K, Haxhinasto S, Perez J, Melamed R et al. Foxp3 transcription-factor-dependent and -independent regulation of the regulatory T cell transcriptional signature. Immunity 2007; 27(5): 786-800.
47. Feng Y, van der Veeken J, Shugay M, Putintseva EV, Osmanbeyoglu HU, Dikiy S et al. A mechanism for expansion of regulatory T-cell repertoire and its role in self-tolerance. Nature 2015; 528(7580): 132-6.
48. Takahashi R, Yoshimura A. SOCS1 and regulation of regulatory T cells plasticity. J Immunol Res 2014; 943149(10): 15.
49. Li X, Liang Y, LeBlanc M, Benner C, Zheng Y. Function of a Foxp3 cis-Element in Protecting Regulatory T Cell Identity. Cell 2014; 158(4): 734-48.
50. Mao K, Chen S, Chen M, Ma Y, Wang Y, Huang B et al. Nitric oxide suppresses NLRP3 inflammasome activation and protects against LPS-induced septic shock. Cell Res 2013; 23(2): 201-12.
51. Tone Y, Furuuchi K, Kojima Y, Tykocinski ML, Greene MI, Tone M. Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer. Nat Immunol 2008; 9(2): 194-202.
52. Xu L, Kitani A, Stuelten C, McGrady G, Fuss I, Strober W. Positive and negative transcriptional regulation of the Foxp3 gene is mediated by access and binding of the Smad3 protein to enhancer I. Immunity 2010; 33(3): 313-25.
53. Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y et al. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. Nature 2012; 482(7385): 395-9.
54. Feuerer M, Hill JA, Kretschmer K, von Boehmer H, Mathis D, Benoist C. Genomic definition of multiple ex vivo regulatory T cell subphenotypes. Proceedings of the National Academy of Sciences of the United States of America 2010; 107(13): 5919-24.
55. Morikawa H, Ohkura N, Vandenbon A, Itoh M, Nagao-Sato S, Kawaji H et al. Differential roles of epigenetic changes and Foxp3 expression in regulatory T cell-specific transcriptional regulation. Proceedings of the National Academy of Sciences of the United States of America 2014; 111(14): 5289-94.
56. Ohkura N, Hamaguchi M, Morikawa H, Sugimura K, Tanaka A, Ito Y et al. T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development. Immunity 2012; 37(5): 785-99.
57. Feng Y, Arvey A, Chinen T, van der Veeken J, Gasteiger G, Rudensky AY. Control of the Inheritance of Regulatory T Cell Identity by a cis Element in the Foxp3 Locus. Cell 2014; 158(4): 749-63.
58. Ono M, Yaguchi H, Ohkura N, Kitabayashi I, Nagamura Y, Nomura T et al. Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. Nature 2007; 446(7136): 685-9.
59. Yang BH, Hagemann S, Mamareli P, Lauer U, Hoffmann U, Beckstette M et al. Foxp3(+) T cells expressing RORgammat represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. Mucosal immunology 2016; 9(2): 444-57.
60. Stritesky GL, Yeh N, Kaplan MH. IL-23 promotes maintenance but not commitment to the Th17 lineage. J Immunol 2008; 181(9): 5948-55.
61. Levine AG, Arvey A, Jin W, Rudensky AY. Continuous requirement for the TCR in regulatory T cell function. Nature immunology 2014; 15(11): 1070-8.
62. Farache J, Koren I, Milo I, Gurevich I, Kim KW, Zigmond E et al.

Luminal bacteria recruit CD103+ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation. Immunity 2013; 38(3): 581-95.
63. McDole JR, Wheeler LW, McDonald KG, Wang B, Konjufca V, Knoop KA et al. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. Nature 2012; 483(7389): 345-9.
64. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science 2007; 317(5835): 256-60.
65. Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y et al. Increased expression of interleukin 17 in inflammatory bowel disease. Gut 2003; 52(1): 65-70.
66. Izcue A, Hue S, Buonocore S, Arancibia-Cárcamo CV, Ahern PP, Iwakura Y et al. Interleukin-23 restrains regulatory T cell activity to drive T cell-dependent colitis. Immunity 2008; 28(4): 559-570.
67. Yang XO, Chang SH, Park H, Nurieva R, Shah B, Acero L et al. Regulation of inflammatory responses by IL-17F. Journal of Experimental Medicine 2008; 205(5): 1063-1075.
68. Blaschitz C, Raffatellu M. Th17 cytokines and the gut mucosal barrier. Journal of clinical immunology 2010; 30(2): 196-203.
69. Kleinewietfeld M, Hafler DA. The plasticity of human Treg and Th17 cells and its role in autoimmunity. Seminars in immunology 2013; 25(4): 305-12.
70. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annual review of immunology 2009; 27: 485-517.
71. Luchtman DW, Ellwardt E, Larochelle C, Zipp F. IL-17 and related cytokines involved in the pathology and immunotherapy of multiple sclerosis: current and future developments. Cytokine & growth factor reviews 2014; 25(4): 403-413.
72. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006; 441(7090): 235-238.
73. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFβ in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 2006; 24(2): 179-189.
74. Korn T, Bettelli E, Gao W, Awasthi A, Jäger A, Strom TB et al. IL-21 initiates an alternative pathway to induce proinflammatory TH17 cells. Nature 2007; 448(7152): 484-487.
75. Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature 2007; 448(7152): 480-483.
76. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T et al. IL-6 programs TH-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nature immunology 2007; 8(9): 967-974.
77. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ et al. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 2006; 126(6): 1121-1133.
78. Tsukamoto H, Senju S, Matsumura K, Swain SL, Nishimura Y. IL-6-mediated environmental conditioning of defective Th1 differentiation dampens antitumour immune responses in old age. Nature communications 2015; 6: 6702.
79. Xu J, Yang Y, Qiu G, Lal G, Wu Z, Levy DE et al. c-Maf regulates IL-10 expression during Th17 polarization. J Immunol 2009; 182(10): 6226-36.
80. Jain R, Chen Y, Kanno Y, Joyce-Shaikh B, Vahedi G, Hirahara K et al. Interleukin-23-induced transcription factor Blimp-1 promotes pathogenicity of T helper 17 cells. Immunity 2016; 44(1): 131-142.
81. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T et al. TGF-β and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain TH-17 cell–mediated pathology. Nature immunology 2007; 8(12): 1390-1397.
82. McGeachy MJ, Chen Y, Tato CM, Laurence A, Joyce-Shaikh B, Blumenschein WM et al. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17–producing effector T helper cells in vivo. Nature immunology 2009; 10(3): 314-324.
83. Lee Y, Awasthi A, Yosef N, Quintana FJ, Xiao S, Peters A et al. Induction and molecular signature of pathogenic TH17 cells. Nature immunology 2012; 13(10): 991-999.
84. Ghoreschi K, Laurence A, Yang X-P, Tato CM, McGeachy MJ, Konkel JE et al. Generation of pathogenic TH17 cells in the absence of TGF-[beta] signalling. Nature 2010; 467(7318): 967-971.
85. de Beaucoudrey L, Puel A, Filipe-Santos O, Cobat A, Ghandil P, Chrabieh M et al. Mutations in STAT3 and IL12RB1 impair the development of human IL-17–producing T cells. Journal of Experimental Medicine 2008; 205(7): 1543-1550.
86. Liu X, Lee YS, Yu C-R, Egwuagu CE. Loss of STAT3 in CD4+ T cells prevents development of experimental autoimmune diseases. The Journal of Immunology 2008; 180(9): 6070-6076.
87. Harris TJ, Grosso JF, Yen H-R, Xin H, Kortylewski M, Albesiano E et al. Cutting edge: An in vivo requirement for STAT3 signaling in TH17 development and TH17-dependent autoimmunity. The Journal of Immunology 2007; 179(7): 4313-4317.
88. Durant L, Watford WT, Ramos HL, Laurence A, Vahedi G, Wei L et al. Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. Immunity 2010; 32(5): 605-615.
89. Durant L, Watford WT, Ramos HL, Laurence A, Vahedi G, Wei L et al. Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. Immunity 2010; 32(5): 605-15.
90. Ise W, Kohyama M, Schraml BU, Zhang T, Schwer B, Basu U et al. The transcription factor BATF controls the global regulators of class-switch recombination in both B cells and T cells. Nature immunology 2011; 12(6): 536-543.
91. Schraml BU, Hildner K, Ise W, Lee W-L, Smith WA-E, Solomon B et al. The AP-1 transcription factor Batf controls TH17 differentiation. Nature 2009; 460(7253): 405-409.
92. Brüstle A, Heink S, Huber M, Rosenplänter C, Stadelmann C, Yu P et al. The development of inflammatory TH-17 cells requires interferon-regulatory factor 4. Nature immunology 2007; 8(9): 958-966.
93. Li P, Spolski R, Liao W, Wang L, Murphy TL, Murphy KM et al. BATF-JUN is critical for IRF4-mediated transcription in T cells. Nature 2012; 490(7421): 543-546.
94. Ciofani M, Madar A, Galan C, Sellars M, Mace K, Pauli F et al. A validated regulatory network for Th17 cell specification. Cell 2012; 151(2): 289-303.
95. Dang EV, Barbi J, Yang H-Y, Jinasena D, Yu H, Zheng Y et al. Control of T H 17/T reg balance by hypoxia-inducible factor 1. Cell 2011; 146(5): 772-784.
96. Collins A, Littman DR, Taniuchi I. RUNX proteins in transcription factor networks that regulate T-cell lineage choice. Nature reviews Immunology 2009; 9(2): 106-115.
97. Zhang F, Meng G, Strober W. Interactions among the transcription factors Runx1, RORγt and Foxp3 regulate the differentiation of interleukin 17–producing T cells. Nature immunology 2008; 9(11): 1297-1306.
98. Esser C, Rannug A, Stockinger B. The aryl hydrocarbon receptor in immunity. Trends in immunology 2009; 30(9): 447-454.
99. Beischlag TV, Morales JL, Hollingshead BD, Perdew GH. The aryl hydrocarbon receptor complex and the control of gene expression. Critical Reviews™ in Eukaryotic Gene Expression 2008; 18(3).
100. Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L,

Renauld J-C et al. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 2008; 453(7191): 106-109.
101. Veldhoen M, Hirota K, Christensen J, O'Garra A, Stockinger B. Natural agonists for aryl hydrocarbon receptor in culture medium are essential for optimal differentiation of Th17 T cells. Journal of Experimental Medicine 2009; 206(1): 43-49.
102. Liu XK, Lin X, Gaffen SL. Crucial role for nuclear factor of activated T cells in T cell receptor-mediated regulation of human interleukin-17. Journal of Biological Chemistry 2004; 279(50): 52762-52771.
103. Gomez-Rodriguez J, Sahu N, Handon R, Davidson TS, Anderson SM, Kirby MR et al. Differential expression of interleukin-17A and-17F is coupled to T cell receptor signaling via inducible T cell kinase. Immunity 2009; 31(4): 587-597.
104. Ghosh S, Koralov SB, Stevanovic I, Sundrud MS, Sasaki Y, Rajewsky K et al. Hyperactivation of nuclear factor of activated T cells 1 (NFAT1) in T cells attenuates severity of murine autoimmune encephalomyelitis. Proceedings of the National Academy of Sciences 2010; 107(34): 15169-15174.
105. Maynard CL, Harrington LE, Janowski KM, Oliver JR, Zindl CL, Rudensky AY et al. Regulatory T cells expressing interleukin 10 develop from Foxp3+ and Foxp3- precursor cells in the absence of interleukin 10. Nature immunology 2007; 8(9): 931-41.
106. Nakamura K, Kitani A, Strober W. Cell contact–dependent immunosuppression by CD4+ CD25+ regulatory T cells is mediated by cell surface–bound transforming growth factor β. Journal of Experimental Medicine 2001; 194(5): 629-644.
107. Das J, Ren G, Zhang L, Roberts AI, Zhao X, Bothwell AL et al. Transforming growth factor β is dispensable for the molecular orchestration of Th17 cell differentiation. Journal of Experimental Medicine 2009; 206(11): 2407-2416.
108. Brucklacher-Waldert V, Carr EJ, Linterman MA, Veldhoen M. Cellular Plasticity of CD4+ T Cells in the Intestine. Frontiers in immunology 2014; 5: 488.
109. Kulkarni N, Meitei HT, Sonar SA, Sharma PK, Mujeeb VR, Srivastava S et al. CCR6 signaling inhibits suppressor function of induced-Treg during gut inflammation. J Autoimmun 2018; 88:121-130.
110. Sethi A, Kulkarni N, Sonar S, Lal G. Role of miRNAs in CD4 T cell plasticity during inflammation and tolerance. Front Genet 2013; 4: 8.
111. Xu L, Kitani A, Fuss I, Strober W. Cutting edge: regulatory T cells induce CD4+ CD25− Foxp3− T cells or are self-induced to become Th17 cells in the absence of exogenous TGF-β. The Journal of Immunology 2007; 178(11): 6725-6729.
112. Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP et al. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity 2008; 29(1): 44-56.
113. Lal G, Yin N, Xu J, Lin M, Schroppel S, Ding Y et al. Distinct inflammatory signals have physiologically divergent effects on epigenetic regulation of foxp3 expression and treg function. Am J Transplant 2011; 11(2): 203-14.
114. Koenen HJ, Smeets RL, Vink PM, Van Rijssen E, Boots AM, Joosten I. Human CD25highFoxp3pos regulatory T cells differentiate into IL-17–producing cells. Blood 2008; 112(6): 2340-2352.
115. Beriou G, Costantino CM, Ashley CW, Yang L, Kuchroo VK, Baecher-Allan C et al. IL-17–producing human peripheral regulatory T cells retain suppressive function. Blood 2009; 113(18): 4240-4249.
116. Komatsu N, Okamoto K, Sawa S, Nakashima T, Oh-hora M, Kodama T et al. Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis. Nat Med 2014; 20(1): 62-8.
117. Ueno A, Jijon H, Chan R, Ford K, Hirota C, Kaplan GG et al. Increased prevalence of circulating novel IL-17 secreting Foxp3 expressing CD4+ T cells and defective suppressive function of circulating Foxp3+ regulatory cells support plasticity between Th17 and regulatory T cells in inflammatory bowel disease patients. Inflammatory bowel diseases 2013; 19(12): 2522-2534.
118. Hovhannisyan Z, Treatman J, Littman DR, Mayer L. Characterization of interleukin-17–producing regulatory T cells in inflamed intestinal mucosa from patients with inflammatory bowel diseases. Gastroenterology 2011; 140(3): 957-965.
119. DuPage M, Bluestone JA. Harnessing the plasticity of CD4(+) T cells to treat immune-mediated disease. Nat Rev Immunol 2016; 16(3): 149-63.
120. Ardizzone S, Bevivino G, Monteleone G. Mongersen, an oral Smad7 antisense oligonucleotide, in patients with active Crohn's disease. Therap Adv Gastroenterol 2016; 9(4): 527-32.
121. Monteleone G, Neurath MF, Ardizzone S, Di Sabatino A, Fantini MC, Castiglione F et al. Mongersen, an oral SMAD7 antisense oligonucleotide, and Crohn's disease. The New England journal of medicine 2015; 372(12): 1104-13.
122. Coskun M, Vermeire S, Nielsen OH. Novel Targeted Therapies for Inflammatory Bowel Disease. Trends Pharmacol Sci 2017; 38(2): 127-142.
123. Quintana FJ, Jin H, Burns EJ, Nadeau M, Yeste A, Kumar D et al. Corrigendum: aiolos promotes TH17 differentiation by directly silencing Il2 expression. Nature immunology 2014; 15(1): 109.
124. Raffin C, Pignon P, Celse C, Debien E, Valmori D, Ayyoub M. Human memory Helios− FOXP3+ regulatory T cells (Tregs) encompass induced Tregs that express Aiolos and respond to IL-1β by downregulating their suppressor functions. The Journal of Immunology 2013; 191(9): 4619-4627.
125. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 2012; 491(7422): 119-24.
126. Basu R, Whitley SK, Bhaumik S, Zindl CL, Schoeb TR, Benveniste EN et al. IL-1 signaling modulates activation of STAT transcription factors to antagonize retinoic acid signaling and control the TH17 cell-iTreg cell balance. Nature immunology 2015; 16(3): 286-95.
127. Bonovas S, Lytras T, Nikolopoulos G, Peyrin-Biroulet L, Danese S. Systematic review with network meta-analysis: comparative assessment of tofacitinib and biological therapies for moderate-to-severe ulcerative colitis. Alimentary pharmacology & therapeutics 2017.
128. Argollo M, Fiorino G, Hindryckx P, Peyrin-Biroulet L, Danese S. Novel therapeutic targets for inflammatory bowel disease. J Autoimmun 2017; 85: 103-116.
129. Olivera P, Danese S, Peyrin-Biroulet L. Next generation of small molecules in inflammatory bowel disease. Gut 2017; 66(2): 199-209.
130. Panes J, Vermeire S, Lindsay JO, Sands BE, Su C, Friedman G et al. Tofacitinib in Patients with Ulcerative Colitis: Health-Related Quality of Life in Phase 3 Randomised Controlled Induction and Maintenance Studies. J Crohns Colitis 2018; 12(2): 145-156.
131. Vermeire S, Schreiber S, Petryka R, Kuehbacher T, Hebuterne X, Roblin X et al. Clinical remission in patients with moderate-to-severe Crohn's disease treated with filgotinib (the FITZROY study): results from a phase 2, double-blind, randomised, placebo-controlled trial. Lancet 2017; 389(10066): 266-275.
132. Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B et al. Phenotypic and functional features of human Th17 cells. Journal of Experimental Medicine 2007; 204(8): 1849-1861.

133. Acosta-Rodriguez EV, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A et al. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. Nat Immunol 2007; 8(6): 639-46.
134. Lee YK, Turner H, Maynard CL, Oliver JR, Chen D, Elson CO et al. Late developmental plasticity in the T helper 17 lineage. Immunity 2009; 30(1): 92-107.
135. Harbour SN, Maynard CL, Zindl CL, Schoeb TR, Weaver CT. Th17 cells give rise to Th1 cells that are required for the pathogenesis of colitis. Proceedings of the National Academy of Sciences 2015; 112(22): 7061-7066.
136. D'Haens G, Sandborn WJ, Colombel JF, Rutgeerts P, Brown K, Barkay H et al. A phase II study of laquinimod in Crohn's disease. Gut 2015; 64(8): 1227-35.
137. Hueber W, Sands BE, Lewitzky S, Vandemeulebroecke M, Reinisch W, Higgins PD et al. Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. Gut 2012; 61(12): 1693-700.
138. Targan SR, Feagan B, Vermeire S, Panaccione R, Melmed GY, Landers C et al. A Randomized, Double-Blind, Placebo-Controlled Phase 2 Study of Brodalumab in Patients With Moderate-to-Severe Crohn's Disease. Am J Gastroenterol 2016; 111(11): 1599-1607.
139. Kaser A. Not all monoclonals are created equal - lessons from failed drug trials in Crohn's disease. Best Pract Res Clin Gastroenterol 2014; 28(3): 437-49.
140. Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B et al. Phenotypic and functional features of human Th17 cells. J Exp Med 2007; 204(8): 1849-61.
141. Ueno A, Jijon H, Chan R, Ford K, Hirota C, Kaplan GG et al. Increased prevalence of circulating novel IL-17 secreting Foxp3 expressing CD4+ T cells and defective suppressive function of circulating Foxp3+ regulatory cells support plasticity between Th17 and regulatory T cells in inflammatory bowel disease patients. Inflammatory bowel diseases 2013; 19(12): 2522-34.
142. Hovhannisyan Z, Treatman J, Littman DR, Mayer L. Characterization of interleukin-17-producing regulatory T cells in inflamed intestinal mucosa from patients with inflammatory bowel diseases. Gastroenterology 2011; 140(3): 957-65.
143. Sonar SA, Lal G. Differentiation and Transmigration of CD4 T Cells in Neuroinflammation and Autoimmunity. Frontiers in immunology 2017; 8: 1695.
144. Wu C, Yosef N, Thalhamer T, Zhu C, Xiao S, Kishi Y et al. Induction of pathogenic TH17 cells by inducible salt-sensing kinase SGK1. Nature 2013; 496(7446): 513-7.
145. Lee Y, Awasthi A, Yosef N, Quintana FJ, Xiao S, Peters A et al. Induction and molecular signature of pathogenic TH17 cells. Nat Immunol 2012; 13(10): 991-9.
146. Feagan BG, Sandborn WJ, Gasink C, Jacobstein D, Lang Y, Friedman JR et al. Ustekinumab as Induction and Maintenance Therapy for Crohn's Disease. The New England journal of medicine 2016; 375(20): 1946-1960.
147. Singh S, Kroe-Barrett RR, Canada KA, Zhu X, Sepulveda E, Wu H et al. Selective targeting of the IL23 pathway: Generation and characterization of a novel high-affinity humanized anti-IL23A antibody. MAbs 2015; 7(4): 778-91.
148. Feagan BG, Sandborn WJ, D'Haens G, Panes J, Kaser A, Ferrante M et al. Induction therapy with the selective interleukin-23 inhibitor risankizumab in patients with moderate-to-severe Crohn's disease: a randomised, double-blind, placebo-controlled phase 2 study. Lancet 2017; 389(10080): 1699-1709.
149. Sato W, Aranami T, Yamamura T. Cutting edge: Human Th17 cells are identified as bearing CCR2+CCR5- phenotype. J Immunol 2007; 178(12): 7525-9.
150. Singh SP, Zhang HH, Foley JF, Hedrick MN, Farber JM. Human T cells that are able to produce IL-17 express the chemokine receptor CCR6. J Immunol 2008; 180(1): 214-21.
151. Lim HW, Lee J, Hillsamer P, Kim CH. Human Th17 cells share major trafficking receptors with both polarized effector T cells and FOXP3+ regulatory T cells. J Immunol 2008; 180(1): 122-9.
152. Zhang N, Schroppel B, Lal G, Jakubzick C, Mao X, Chen D et al. Regulatory T cells sequentially migrate from inflamed tissues to draining lymph nodes to suppress the alloimmune response. Immunity 2009; 30(3): 458-69.
153. Wang C, Kang SG, Lee J, Sun Z, Kim CH. The roles of CCR6 in migration of Th17 cells and regulation of effector T-cell balance in the gut. Mucosal immunology 2009; 2(2): 173-83.
154. Lee AY, Eri R, Lyons AB, Grimm MC, Korner H. CC Chemokine Ligand 20 and Its Cognate Receptor CCR6 in Mucosal T Cell Immunology and Inflammatory Bowel Disease: Odd Couple or Axis of Evil? Frontiers in immunology 2013; 4: 194.
155. Yamazaki T, Yang XO, Chung Y, Fukunaga A, Nurieva R, Pappu B et al. CCR6 regulates the migration of inflammatory and regulatory T cells. J Immunol 2008; 181(12): 8391-401.
156. Kulkarni N, Lal G. Non-chemotactic function of chemokine receptor CCR6 in the differentiation and function of Th17 and Treg cells. Cytokine 2016; 87: 156-157.
157. Kulkarni N, Lal G. CCR6 intrinsic signaling promotes Th17 cell differentiation during autoimmunity. Eur J Immunol 2016; 46(Suppl1): 89.
158. Katchar K, Kelly CP, Keates S, O'Brien M J, Keates AC. MIP-3alpha neutralizing monoclonal antibody protects against TNBS-induced colonic injury and inflammation in mice. American journal of physiology 2007; 292(5): G1263-71.
159. Robert R, Juglair L, Lim EX, Ang C, Wang CJH, Ebert G et al. A fully humanized IgG-like bispecific antibody for effective dual targeting of CXCR3 and CCR6. PLoS One 2017; 12(9): e0184278.
160. Getschman AE, Imai Y, Larsen O, Peterson FC, Wu X, Rosenkilde MM et al. Protein engineering of the chemokine CCL20 prevents psoriasiform dermatitis in an IL-23-dependent murine model. Proc Natl Acad Sci U S A 2017; 114(47): 12460-12465.
161. Robert R, Ang C, Sun G, Juglair L, Lim EX, Mason LJ et al. Essential role for CCR6 in certain inflammatory diseases demonstrated using specific antagonist and knockin mice. JCI Insight 2017; 2(15).
162. Nielsen OH, Li Y, Johansson-Lindbom B, Coskun M. Sphingosine-1-Phosphate Signaling in Inflammatory Bowel Disease. Trends in molecular medicine 2017; 23(4): 362-374.
163. Ledgerwood LG, Lal G, Zhang N, Garin A, Esses SJ, Ginhoux F et al. The sphingosine 1-phosphate receptor 1 causes tissue retention by inhibiting the entry of peripheral tissue T lymphocytes into afferent lymphatics. Nat Immunol 2008; 9(1): 42-53.
164. Liu G, Yang K, Burns S, Shrestha S, Chi H. The S1P(1)-mTOR axis directs the reciprocal differentiation of T(H)1 and T(reg) cells. Nat Immunol 2010; 11(11): 1047-56.
165. Peyrin-Biroulet L, Christopher R, Behan D, Lassen C. Modulation of sphingosine-1-phosphate in inflammatory bowel disease. Autoimmunity reviews 2017; 16(5): 495-503.
166. Sandborn WJ, Feagan BG, Wolf DC, D'Haens G, Vermeire S, Hanauer SB et al. Ozanimod Induction and Maintenance Treatment for Ulcerative Colitis. The New England journal of medicine 2016; 374(18): 1754-62.
167. Aroniadis OC, Brandt LJ. Intestinal microbiota and the efficacy of fecal microbiota transplantation in gastrointestinal disease. Gastroenterol Hepatol (N Y) 2014; 10(4): 230-7.
168. Costello SP, Soo W, Bryant RV, Jairath V, Hart AL, Andrews

JM. Systematic review with meta-analysis: faecal microbiota transplantation for the induction of remission for active ulcerative colitis. Alimentary pharmacology & therapeutics 2017; 46(3): 213-224.
169. Moayyedi P, Surette MG, Kim PT, Libertucci J, Wolfe M, Onischi C et al. Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. Gastroenterology 2015; 149(1): 102-109 e6.
170. Rossen NG, Fuentes S, van der Spek MJ, Tijssen JG, Hartman JH, Duflou A et al. Findings From a Randomized Controlled Trial of Fecal Transplantation for Patients With Ulcerative Colitis. Gastroenterology 2015; 149(1): 110-118 e4.
171. Paramsothy S, Kamm MA, Kaakoush NO, Walsh AJ, van den Bogaerde J, Samuel D et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. Lancet 2017; 389(10075): 1218-1228.
172. Netz U, Carter J, Eichenberger MR, Feagins K, Galbraith NJ, Dryden GW et al. Plasma microRNA Profile Differentiates Crohn's Colitis From Ulcerative Colitis. Inflammatory bowel diseases 2017; 24(1): 159-165.
173. Schaefer JS, Attumi T, Opekun AR, Abraham B, Hou J, Shelby H et al. MicroRNA signatures differentiate Crohn's disease from ulcerative colitis. BMC immunology 2015; 16: 5.
174. Paraskevi A, Theodoropoulos G, Papaconstantinou I, Mantzaris G, Nikiteas N, Gazouli M. Circulating MicroRNA in inflammatory bowel disease. J Crohns Colitis 2012; 6(9): 900-4.
175. Pacholewska A, Kraft MF, Gerber V, Jagannathan V. Differential Expression of Serum MicroRNAs Supports CD4(+) T Cell Differentiation into Th2/Th17 Cells in Severe Equine Asthma. Genes (Basel) 2017; 8(12).
176. Li B, Wang X, Choi IY, Wang YC, Liu S, Pham AT et al. miR-146a modulates autoreactive Th17 cell differentiation and regulates organ-specific autoimmunity. J Clin Invest 2017; 127(10): 3702-3716.
177. Ananthakrishnan AN, Bernstein CN, Iliopoulos D, Macpherson A, Neurath MF, Ali RAR et al. Environmental triggers in IBD: a review of progress and evidence. Nat Rev Gastroenterol Hepatol 2018; 15(1): 39-49.
178. Danese S, Fiocchi C. Ulcerative colitis. The New England journal of medicine 2011; 365(18): 1713-25.
179. Baumgart DC, Sandborn WJ. Crohn's disease. Lancet 2012; 380(9853): 1590-605.
180. Neurath MF. Cytokines in inflammatory bowel disease. Nature reviews. Immunology 2014; 14(5): 329-42.
181. van der Heide F, Dijkstra A, Weersma RK, Albersnagel FA, van der Logt EM, Faber KN et al. Effects of active and passive smoking on disease course of Crohn's disease and ulcerative colitis. Inflammatory bowel diseases 2009; 15(8): 1199-207.
182. Bastida G, Beltran B. Ulcerative colitis in smokers, non-smokers and ex-smokers. World journal of gastroenterology : WJG 2011; 17(22): 2740-7.
183. Andersson RE, Olaison G, Tysk C, Ekbom A. Appendectomy and protection against ulcerative colitis. The New England journal of medicine 2001; 344(11): 808-14.
184. Kaplan GG, Jackson T, Sands BE, Frisch M, Andersson RE, Korzenik J. The risk of developing Crohn's disease after an appendectomy: a meta-analysis. The American journal of gastroenterology 2008; 103(11): 2925-31.
185. Hou JK, Abraham B, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. The American journal of gastroenterology 2011; 106(4): 563-73.
186. Sakamoto N, Kono S, Wakai K, Fukuda Y, Satomi M, Shimoyama T et al. Dietary risk factors for inflammatory bowel disease: a multicenter case-control study in Japan. Inflammatory bowel diseases 2005; 11(2): 154-63.
187. Fuss IJ, Neurath M, Boirivant M, Klein JS, de la Motte C, Strong SA et al. Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. J Immunol 1996; 157(3): 1261-70.
188. Heller F, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B et al. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. Gastroenterology 2005; 129(2): 550-64.
189. Yu J, He S, Liu P, Hu Y, Wang L, Wang X et al. Interleukin21 promotes the development of ulcerative colitis and regulates the proliferation and secretion of follicular T helper cells in the colitides microenvironment. Mol Med Rep 2015; 11(2): 1049-56.
190. Sakuraba A, Sato T, Kamada N, Kitazume M, Sugita A, Hibi T. Th1/Th17 immune response is induced by mesenteric lymph node dendritic cells in Crohn's disease. Gastroenterology 2009; 137(5): 1736-45.
191. Sarra M, Monteleone I, Stolfi C, Fantini MC, Sileri P, Sica G et al. Interferon-gamma-expressing cells are a major source of interleukin-21 in inflammatory bowel diseases. Inflammatory bowel diseases 2010; 16(8): 1332-9.
192. Gerlach K, Hwang Y, Nikolaev A, Atreya R, Dornhoff H, Steiner S et al. TH9 cells that express the transcription factor PU.1 drive T cell-mediated colitis via IL-9 receptor signaling in intestinal epithelial cells. Nat Immunol 2014; 15(7): 676-86.
193. Leung JM, Davenport M, Wolff MJ, Wiens KE, Abidi WM, Poles MA et al. IL-22-producing CD4+ cells are depleted in actively inflamed colitis tissue. Mucosal immunology 2014; 7(1): 124-33.
194. Neurath MF, Weigmann B, Finotto S, Glickman J, Nieuwenhuis E, Iijima H et al. The transcription factor T-bet regulates mucosal T cell activation in experimental colitis and Crohn's disease. The Journal of experimental medicine 2002; 195(9): 1129-43.
195. Takayama T, Kamada N, Chinen H, Okamoto S, Kitazume MT, Chang J et al. Imbalance of NKp44(+)NKp46(-) and NKp44(-)NKp46(+) natural killer cells in the intestinal mucosa of patients with Crohn's disease. Gastroenterology 2010; 139(3): 882-92, 892 e1-3.
196. Geremia A, Arancibia-Carcamo CV, Fleming MP, Rust N, Singh B, Mortensen NJ et al. IL-23-responsive innate lymphoid cells are increased in inflammatory bowel disease. J Exp Med 2011; 208(6): 1127-33.
197. Pastorelli L, Garg RR, Hoang SB, Spina L, Mattioli B, Scarpa M et al. Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis and in experimental Th1/Th2 driven enteritis. Proceedings of the National Academy of Sciences of the United States of America 2010; 107(17): 8017-22.
198. Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A et al. IL-33 is a crucial amplifier of innate rather than acquired immunity. Proceedings of the National Academy of Sciences of the United States of America 2010; 107(43): 18581-6.
199. McNamee EN, Masterson JC, Jedlicka P, McManus M, Grenz A, Collins CB et al. Interleukin 37 expression protects mice from colitis. Proceedings of the National Academy of Sciences of the United States of America 2011; 108(40): 16711-6.
200. Danese S, Rudzinski J, Brandt W, Dupas JL, Peyrin-Biroulet L, Bouhnik Y et al. Tralokinumab for moderate-to-severe UC: a randomised, double-blind, placebo-controlled, phase IIa study. Gut 2015; 64(2): 243-9.
201. Spiess C, Bevers J, 3rd, Jackman J, Chiang N, Nakamura G, Dillon

M et al. Development of a human IgG4 bispecific antibody for dual targeting of interleukin-4 (IL-4) and interleukin-13 (IL-13) cytokines. The Journal of biological chemistry 2013; 288(37): 26583-93.
202. Reinisch W, de Villiers W, Bene L, Simon L, Racz I, Katz S et al. Fontolizumab in moderate to severe Crohn's disease: a phase 2, randomized, double-blind, placebo-controlled, multiple-dose study. Inflammatory bowel diseases 2010; 16(2): 233-42.
203. Halloran B, Chang J, Shih DQ, McGovern D, Famulski K, Evaschesen C et al. Molecular patterns in human ulcerative colitis and correlation with response to infliximab. Inflammatory bowel diseases 2014; 20(12): 2353-63.
204. Schreiber S, Fedorak RN, Nielsen OH, Wild G, Williams CN, Nikolaus S et al. Safety and efficacy of recombinant human interleukin 10 in chronic active Crohn's disease. Crohn's Disease IL-10 Cooperative Study Group. Gastroenterology 2000; 119(6): 1461-72.
205. Monteleone G, Fantini MC, Onali S, Zorzi F, Sancesario G, Bernardini S et al. Phase I clinical trial of Smad7 knockdown using antisense oligonucleotide in patients with active Crohn's disease. Molecular therapy : the journal of the American Society of Gene Therapy 2012; 20(4): 870-6.
206. Sandborn WJ, Gasink C, Gao LL, Blank MA, Johanns J, Guzzo C et al. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. The New England journal of medicine 2012; 367(16): 1519-28.