Interleukin 23 in Crohn’s Disease

Ahmet Eken, PhD,* Akhilesh K. Singh, PhD,* and Mohamed Oukka, PhD†

Abstract: Crohn’s disease (CD) is a lifelong inflammatory condition with underlying environmental and genetic components. CD affects multiple parts of the gastrointestinal tract, and it has a growing incidence in Western societies. IL-23 receptor variants have been identified as susceptibility or resistance factors for CD in genome-wide association studies. Accordingly, IL-23 is required for the development of experimental inflammatory bowel disease in many murine models. IL-23 receptor is expressed by both innate and adaptive immune cells, which include Th17, natural killer T, γδ T cells, and ROrientia sp. innate lymphoid cells all of which are capable of secreting IL-17A, IL-17F, IL-22, and interferon-γ. Although the role of IL-23 is indisputably established in disease pathogenesis, recent studies have shown that innate lymphoid cells contribute to disease development. In this review, we have summarized and discussed these findings with an emphasis not only on the contribution of Th17 but also on innate lymphoid cells to disease etiology.

(IL-23 cytokine and signaling)

IL-23 cytokine and signaling

Innate IL-23 is a heterodimeric cytokine composed of a p40 subunit shared by IL-12 and a specific p19 subunit. IL-23 is ubiquitously expressed in the terminal ileum of mice in a microbiotadependent fashion; such as germ-free mice have drastically reduced levels of IL-23. Mainly, professional antigen-presenting cells, that is, dendritic cells (DC) and macrophages, and monocytes produce IL-23 in response to a variety of bacterial and fungal pathogen-associated molecular patterns and costimulatory molecule ligation. Pattern recognition receptors, TLR2 and NOD-like receptors together, or NOD2 receptor alone, TLR2, 4, 6, 9, C type lectin receptors, and costimulatory molecule CD40, when activated by their corresponding ligands, promote production of IL-23 and IL-1β, which contributes to Th17-polarizing milieu. Distinct subsets of DCs were defined in the murine intestine. CD11b+CD103+ subset, although not exclusive, seems to be the major producer of IL-23 during colitis development or host protective responses. In both adaptive and innate murine models of inflammatory bowel disease, CD11c+ DCs were demonstrated to produce large quantities of IL-23. Accordingly, elevated levels of IL-23 in the intestinal biopsies of patients with Crohn’s disease (CD) and ulcerative colitis (UC) have been reported by several investigators, and the cellular sources of IL-23 were determined as CD68+CD14+ DCs and macrophages. Specific ablation of CD11c+ cells in mice ameliorates dextran sodium sulfate (DSS)-induced colitis, pointing to the importance of this cellular source in IBD pathogenesis.

An essential role for IL-23 in IBD pathogenesis has been established by genetic studies in mice. IL-23 transgenic mice develop systemic inflammation including enterocolitis. By using p19+/−, p35−/−, and p40−/− mice and neutralizing antibodies specific to p19, p35 and p40, IL-23p19, but not IL-12p35, was shown to be required for the development of colitis in various mouse models, including innate cell–driven colitis induced by Helicobacter hepaticus in both Rag−/− or Rag-sufficient hosts and adaptive colitis induced in Rag−/− mice by the transfer of CD45RBhigh-naive T cells or CD45RBlow IL-10−/− memory T cells, and spontaneous colitis that develops in il-10−/− mice. Additionally, p19 knockout (p19KO) mice are resistant to the development of chemically induced colitis by means of DSS treatment. Although the role of IL-23 is indisputably established by these studies in various contexts, the involvement of IL-12/STAT4 axis and Th1 cells cannot entirely be discredited in some murine colitis models. In this regard, studies show that transgenic STAT4 mice develop colitis, and p19KO mice are more susceptible to 2,4,6-trinitrobenzenesulfonic acid (TNBS)–induced colitis with elevated IL-12 expression and Th1 cytokine signature. IL-23 signals through its heterodimeric receptor which are composed of IL-23 receptor (IL-23R) and IL-12Rβ1. IL-23R is associated with JAK2, and on ligand binding, it dimerizes with beta subunit and activates JAK2/TYK2 and STAT 1, 2, 4 but predominantly STAT3. On IL-23 stimulation, NFκB and PI3K/Akt pathways are activated in parallel, activated STAT3 is further phosphorylated by PI3K/Akt, and these 2 parallel pathways were shown to regulate IL-17 gene expression. Although P38 MAPK pathway is not involved in IL-17 production, MAPK/PI3K has been shown to be activated by IL-23 stimulation. IL-23R green fluorescent protein reporter revealed that a number of these models, including innate cell–driven colitis induced by Helicobacter hepaticus in both Rag−/− or Rag-sufficient hosts and adaptive colitis induced in Rag−/− mice by the transfer of CD45RBhigh-naive T cells or CD45RBlow IL-10−/− memory T cells, and spontaneous colitis that develops in il-10−/− mice. Additionally, p19 knockout (p19KO) mice are resistant to the development of chemically induced colitis by means of DSS treatment. Although the role of IL-23 is indisputably established by these studies in various contexts, the involvement of IL-12/STAT4 axis and Th1 cells cannot entirely be discredited in some murine colitis models. In this regard, studies show that transgenic STAT4 mice develop colitis, and p19KO mice are more susceptible to 2,4,6-trinitrobenzenesulfonic acid (TNBS)–induced colitis with elevated IL-12 expression and Th1 cytokine signature. IL-23 signals through its heterodimeric receptor which are composed of IL-23 receptor (IL-23R) and IL-12Rβ1. IL-23R is associated with JAK2, and on ligand binding, it dimerizes with beta subunit and activates JAK2/TYK2 and STAT 1, 2, 4 but predominantly STAT3. On IL-23 stimulation, NFκB and PI3K/Akt pathways are activated in parallel, activated STAT3 is further phosphorylated by PI3K/Akt, and these 2 parallel pathways were shown to regulate IL-17 gene expression. Although P38 MAPK pathway is not involved in IL-17 production, MAPK/PI3K has been shown to be activated by IL-23 stimulation. IL-23R green fluorescent protein reporter revealed that a number of these models, including innate cell–driven colitis induced by Helicobacter hepaticus in both Rag−/− or Rag-sufficient hosts and adaptive colitis induced in Rag−/− mice by the transfer of CD45RBhigh-naive T cells or CD45RBlow IL-10−/− memory T cells, and spontaneous colitis that develops in il-10−/− mice. Additionally, p19 knockout (p19KO) mice are resistant to the development of chemically induced colitis by means of DSS treatment. Although the role of IL-23 is indisputably established by these studies in various contexts, the involvement of IL-12/STAT4 axis and Th1 cells cannot entirely be discredited in some murine colitis models. In this regard, studies show that transgenic STAT4 mice develop colitis, and p19KO mice are more susceptible to 2,4,6-trinitrobenzenesulfonic acid (TNBS)–induced colitis with elevated IL-12 expression and Th1 cytokine signature. IL-23 signals through its heterodimeric receptor which are composed of IL-23 receptor (IL-23R) and IL-12Rβ1. IL-23R is associated with JAK2, and on ligand binding, it dimerizes with beta subunit and activates JAK2/TYK2 and STAT 1, 2, 4 but predominantly STAT3. On IL-23 stimulation, NFκB and PI3K/Akt pathways are activated in parallel, activated STAT3 is further phosphorylated by PI3K/Akt, and these 2 parallel pathways were shown to regulate IL-17 gene expression. Although P38 MAPK pathway is not involved in IL-17 production, MAPK/PI3K has been shown to be activated by IL-23 stimulation. IL-23R green fluorescent protein reporter revealed that a number of these models, including innate cell–driven colitis induced by Helicobacter hepaticus in both Rag−/− or Rag-sufficient hosts and adaptive colitis induced in Rag−/− mice by the transfer of CD45RBhigh-naive T cells or CD45RBlow IL-10−/− memory T cells, and spontaneous colitis that develops in il-10−/− mice. Additionally, p19 knockout (p19KO) mice are resistant to the development of chemically induced colitis by means of DSS treatment. Although the role of IL-23 is indisputably established by these studies in various contexts, the involvement of IL-12/STAT4 axis and Th1 cells cannot entirely be discredited in some murine colitis models. In this regard, studies show that transgenic STAT4 mice develop colitis, and p19KO mice are more susceptible to 2,4,6-trinitrobenzenesulfonic acid (TNBS)–induced colitis with elevated IL-12 expression and Th1 cytokine signature. IL-23 signals through its heterodimeric receptor which are composed of IL-23 receptor (IL-23R) and IL-12Rβ1. IL-23R is associated with JAK2, and on ligand binding, it dimerizes with beta subunit and activates JAK2/TYK2 and STAT 1, 2, 4 but predominantly STAT3. On IL-23 stimulation, NFκB and PI3K/Akt pathways are activated in parallel, activated STAT3 is further phosphorylated by PI3K/Akt, and these 2 parallel pathways were shown to regulate IL-17 gene expression. Although P38 MAPK pathway is not involved in IL-17 production, MAPK/PI3K has been shown to be activated by IL-23 stimulation. IL-23R green fluorescent protein reporter revealed that a number of these models, including innate cell–driven colitis induced by Helicobacter hepaticus in both Rag−/− or Rag-sufficient hosts and adaptive colitis induced in Rag−/− mice by the transfer of CD45RBhigh-naive T cells or CD45RBlow IL-10−/− memory T cells, and spontaneous colitis that develops in il-10−/− mice. Additionally, p19 knockout (p19KO) mice are resistant to the development of chemically induced colitis by means of DSS treatment. Although the role of IL-23 is indisputably established by these studies in various contexts, the involvement of IL-12/STAT4 axis and Th1 cells cannot entirely be discredited in some murine colitis models. In this regard, studies show that transgenic STAT4 mice develop colitis, and p19KO mice are more susceptible to 2,4,6-trinitrobenzenesulfonic acid (TNBS)–induced colitis with elevated IL-12 expression and Th1 cytokine signature. IL-23 signals through its heterodimeric receptor which are composed of IL-23 receptor (IL-23R) and IL-12Rβ1. IL-23R is associated with JAK2, and on ligand binding, it dimerizes with beta subunit and activates JAK2/TYK2 and STAT 1, 2, 4 but predominantly STAT3. On IL-23 stimulation, NFκB and PI3K/Akt pathways are activated in parallel, activated STAT3 is further phosphorylated by PI3K/Akt, and these 2 parallel pathways were shown to regulate IL-17 gene expression. Although P38 MAPK pathway is not involved in IL-17 production, MAPK/PI3K has been shown to be activated by IL-23 stimulation. IL-23R green fluorescent protein reporter revealed that a number of these models, including innate cell–driven colitis induced by Helicobacter hepaticus in both Rag−/−
of adaptive and innate immune cells express this receptor. Th17, natural killer (NK) T, γδ T cells, group 3 innate lymphoid cells (ILC), DCs, macrophages, and, recently, neutrophils have been shown to express IL-23R and respond to IL-23 stimulation. Much of our knowledge regarding the regulation of IL-23R expression in these cells comes from Th17 cell differentiation studies. Naive T cells, which are negative for IL-23R, upregulate IL-23R expression after stimulation with IL-6 or IL-21 in a STAT3-dependent manner. IL-1β can also induce some IL-23R expression, which is further enhanced by IL-6. IL-23 promotes its own expression in an autocrine fashion. Although required for Th17 differentiation, transforming growth factor (TGF)-β is refractory to IL-23R production. RORγt also promotes IL-23R expression; as such, RORγt-naive T cells upregulates IL-23R minimally compared with wild-type (WT) cells. These inductive pathways of IL-23R expression may apply to other IL-23R+ non-T cells in the IBD settings. Indeed, a recent report revealed that IL-1β can augment IL-23R expression in ILC3; by doing so, it conditions and perhaps contributes to the expansion of these cells, which were demonstrated to be necessary for the pathogenesis in H. hepaticus–induced colitis. Functional IL-23 signaling bears important consequences. Although not required for the initial differentiation, IL-23 can amplify Th17 differentiation with IL-6 and IL-1β in the presence or absence of TGF-β by upregulating RORγt, IL-23R, and IL-1βR expression. IL-23 is important for the pathogenicity of Th17 cells at least in the context of experimental autoimmune encephalomyelitis. Naive T cells differentiated in the presence of IL-23 without TGF-β, called Th17γδ T cells, express T-bet unlike conventional Th17(β) cells differentiated in the presence of TGF-β, and they were shown to home more efficiently to central nervous system, inducing more potent experimental autoimmune encephalomyelitis in mice. IL-23R signaling is also important for the survival of Th17 cells in vivo. This was recently shown to occur, at least partly, as a result of IL-23 dependent upregulation of IL-1βR in Th17 cells in CD45RBhigh T-cell transfer model of colitis. Accordingly, IL-23R−/− T cells were defective in IL-1βR expression, and the transfer of IL-1βR−/−-naive T cells resulted in attenuated colitis with fewer but more apoptotic gut infiltrating Th17 cells.

Besides impacting proinflammatory pathways, IL-23R signaling also dampens the differentiation of induced Treg cell. In the absence of IL-23, adaptively transferred CD4+γδ T cells differentiate more efficiently into Foxp3+ Treg cells in the Rag−/− host gut, which was also confirmed when IL-23R−/−-naive T cells were used for colitis. This is not surprising given the role of IL-23 in Th17 differentiation and the reciprocal regulation between Th17 and Treg fate determination. Furthermore, IL-23, likely in concert with other cytokines produced by DCs, can also promote transition of bona fide Foxp3+ regulatory T cells into a Foxp3+IL-17A+ Th17-like phenotype in an inflammatory milieu, where DCs are polarized to instruct Th17 differentiation. This switch to a proinflammatory program may also have important consequences in the chronic inflammations, such as CD.

On stimulation with IL-23 Th17, NKT, and γδ T cells secrete Th17 signature cytokines IL-17A, IL-17F, IL-22, and IL-21 along with interferon (IFN)-γ. Group 3 innate lymphoid cells and recently neutrophils were also shown to produce IL-17A, IL-17F, and IL-22, although IL-21 is less described. IL-17A and IL-17F can act on both hematopoietic and nonhematopoietic cells; IL-22 acts on nonhematopoietic cells, whereas IL-21R is expressed by hematopoietic cells. These effector cytokines themselves or by means of target cells orchestrate recruitment of leukocytes to local tissues, stimulate production of host protective inflammatory mediators, or initiate repair mechanisms. Through these means, Th17 cytokines have been shown to take either protective or pathogenic roles in IBD pathogenesis in murine models. In the coming sections, this will be reviewed from a cellular perspective.

Although not studied as much as its ligand, IL-23 was shown to be required for the development of naïve CD4+ T cell–induced colitis. Chemically induced DSS-driven colitis is refractory to IL-23R production. Indeed, this was shown to be the case in NOD2−/− mice, which have fewer group 3 ILCs and therefore less IL-22 in the intestines, which in turn results in increased SFB levels. These mice developed spontaneous colitis and aggravated experimental colitis with an increased number of Th17 cells.

Another example suggestive of unrestrained IL-23 responses in CD etiology has come from studies with CD patients with NOD2 mutations. NOD2 is a cytosolic pattern recognition receptor that
detects bacterial cell wall component, and 3 variants of this receptor are found in 40% of CD patients in Western countries. A recent study showed that NOD2 cross talks with TLR2 pathway to regulate IL-23 expression by DCs by means of mobilizing miRNAs. Expression of miR-29 was shown to be augmented by the cross talk of these 2 pathways, which targets directly IL-12p40 messenger RNA and indirectly IL-23p19. DCs with homozygous or heterozygous NOD2 variant 1007insC from CD patients were shown to have defective miR-29 and thus augmented IL-23 expression.\textsuperscript{44}

**TH17 CELLS IN IBD**

**IL-23/IL-17A, IL-17F, and IL-23/IFN-γ Axis**

IL-17A and IL-17F are 2 closely related effector Th17 cytokines produced on IL-23 stimulation of Th17 cells and are recognized by the same receptor complex, IL-17RA and IL-17RB.\textsuperscript{6} IL-17A and IL-17F stimulate the production of chemokines and other inflammatory mediators by epithelial or endothelial cells that recruit neutrophils and other leukocytes to the local tissues.\textsuperscript{46,47} Elevated levels of both IL-17A and IL-17F have been reported in the intestinal biopsies from active CD patients.\textsuperscript{39,48-50} The role of these 2 cytokines has been studied in mouse models. Ogawa et al\textsuperscript{31} have shown that neutralization of IL-17A in DSS model exacerbated colitis, and contrary to the leukocyte-attractive ability of IL-17A, the disease was characterized by higher CD4\textsuperscript{+} T-cell infiltrates and CD11b\textsuperscript{+} granulocyte–monocyte infiltrates. Use of IL-17KO mice recapitulated this phenotype, with higher expression of various leukocytes attracting chemokines CCL2, CCL5, and CCL7.\textsuperscript{52} Along the same lines, adaptive colitis induced by CD45RB\textsuperscript{hi} Foxp3\textsuperscript{−}naive CD4\textsuperscript{+} T cells was shown to occur more aggressively with the transfer of il17r\textsuperscript{−/−} or il17r\textsuperscript{−/−} T cells as compared with WT, and colitis is characterized by similar degrees of cellular infiltration into the gut but with prominent Th1 signature, which was also supported with in vitro experiments demonstrating an inhibitory action of IL-17/IL-17R signaling on Tbx21 expression and Th1-associated transcriptional program within T cells.\textsuperscript{53} Consistent with the protective role of IL17A in these mouse studies, human trials with small-sized CD patients using monoclonal anti-IL-17A antibody, secukinumab, failed to achieve a clinical benefit, and the test group patients experienced exacerbation, adverse effects, and high incidences of fungal infection.\textsuperscript{54}

IL-17FKO mice, on the other hand, developed milder pathology in DSS model compared with WT mice with less leukocyte infiltration to the intestine, indicating nonredundant functions for IL-17A and IL-17F cytokines in this system.\textsuperscript{55} Conversely, IL-17F/−/− CD4\textsuperscript{+}CD25\textsuperscript{−} T cells (naive + memory) can induce comparable, if not more severe, colitis when transferred to Rag\textsuperscript{−/−} hosts, which could partially be suppressed when IL-17A is neutralized implying a redundant and pathogenic function for these closely related cytokines in the adaptive model.\textsuperscript{55} Supporting the proinflammatory role of IL-17A, Yen et al\textsuperscript{13} had demonstrated a pathogenic role for IL-17A together with IL-6 in CD45RB\textsuperscript{hi} CD4\textsuperscript{+} T-cell transfer induced–colitis when T cells lack IL-10. Despite these data, deleting only IL-17A alone in the naive CD4\textsuperscript{+} T cells does not seem to alleviate but perhaps worsen colitis in T-cell transfer models.\textsuperscript{35,53,55,56} Further evidence that may be interpreted as supportive of a proinflammatory role for combined IL-17A and IL-17F came from TNBS-induced colitis model, in which deletion of IL-17RA, the receptor for both IL-17A and IL-17F (but also for IL-17C), or blocking of signaling by means of IL-17RA IgG in WT mice conferred protection against colitis.\textsuperscript{57}

Although IL-17A expression by T cells is not required for naive T cell–driven colitis, RORγ\textsuperscript{t},\textsuperscript{55} STAT3,\textsuperscript{38} IL-23,\textsuperscript{35} and IL-23R,\textsuperscript{36} thus Th17 cells, are required. This suggests the involvement of other Th17-derived cytokines in the pathogenesis. In both CD4\textsuperscript{+} T-cell transfer and H. hepaticus–driven colitis models, IFN-γ’IL-17A’ double-producer Th17 cells were described and were shown to be specifically eliminated when donor T cells lack IL-23R, correlating with the absence of pathology. It is noteworthy that these colitogenic Th17 cells are distinct from naturally occurring Th17 cells in the small intestine in many regards.\textsuperscript{59} Two independent studies showed that these double producers appear only in the colitic mouse gut and proposed that they lose RORγt expression gradually turning into “alternative” Th1 cells through a process blocked by Foxp3\textsuperscript{−} Treg cells and promoted by IL-23 and IL-12.\textsuperscript{56,60,61} It is still not clear whether these Th17/Th1 double producers or alternative Th1 cells are the colitogenic Th1 cells or the conventional Th1 cells that are derived from naive T cells. All of these cells may also contribute to IBD pathology as well. A more recent study by a different group also showed that the frequency of IFN-γ’IL-17A’ Th1/Th17 cells is further increased when there is fewer group 3 innate lymphoid cells in the gut during naive T cell–induced colitis underlining the cross talk between these 2 similar innate and adaptive cell lineages also supporting a role for these double producers in the experimental IBD.\textsuperscript{63}

**IL-23/IL-22 Axis**

IL-22 is a member of the IL-10 family cytokines expressed by Th17 and γδ T cells,\textsuperscript{6} neutrophils,\textsuperscript{57} and ILC3 in response to IL-23 stimulation (see review by Sonnenberg et al\textsuperscript{42}). IL-22 receptor subunit (IL-22R1) is expressed by nonhematopoietic compartment, including epithelial cells in the skin, lung and intestines, and liver and kidney, which are the target cells of IL-22.\textsuperscript{65} IL-22 was shown to stimulate the expression of a number of inflammatory mediators from colonic subepithelial myofibroblast cell lines in vitro and in vivo by other epithelial target cells of chemokines and cytokines. IL-22 is necessary for the host protective immunity against bacterial infections and also for establishing a healthy space/barrier between mucosal commensals and host cells.\textsuperscript{64} IL-22 also serves as a survival and growth factor and is implicated in tissue repair.\textsuperscript{64,65} Elevated levels of IL-22 in mucosal tissue of both UC and CD patients were detected, and CD patients express higher IL-22 than UC patients in both the CD3\textsuperscript{−} and CD3\textsuperscript{+} compartments.\textsuperscript{66} The role of IL-22 in IBD pathogenesis has been questioned as both a protective and pathogenic factor in various mouse models.
A seminal study by Sugimoto et al. demonstrated that IL-22 injections in Tera-/- mice ameliorated the UC-like symptoms. This study revealed an important role for IL-22–induced mucin in IL-22–mediated protection from colitis. Also, the authors showed that the inhibition of IL-22 activity by either a blocking antibody or IL-22 binding protein (IL-22BP) resulted in exacerbated DSS-induced colitis and delayed recovery, which pointed to a potential therapeutic role for IL-22. IL-22 exerts its protective effects through STAT3-mediated pathway in epithelial cells. Accordingly, deletion of STAT3 in intestinal epithelial cells results in defects in the repair mechanisms mobilized by IL-22 during DSS-induced colitis. Although initial study by Leppkes et al. showed that CD4+CD25+ (memory + naive) T cells does not need IL-22 to drive colitis in Rag-/- hosts, transfer of IL-22-expressing CD45RB<sup>hi</sup>Foxp3<sup>-</sup> CD4+ naive T cells resulted in aggravated colitis, which was attributed to the lack of tissue regeneration and repair in the absence of IL-22. Data from our studies indicate that ILC3 also largely contribute to IL-22 production in the inflammatory colons, and in Rag-/-Il-23r-/- hosts, naive T cells can cause more severe pathology when compared with Rag-/- mice, with a major reduction in IL-22 levels supporting these findings. IL-22–mediated protection from colon inflammation can also be recapitulated by mobilizing a parallel IL-22 inductive pathway, using AhR agonist Ficz, in TNBS-induced, DSS-induced, and CD4+-naive T cell–induced colitis models, which result in amelioration in disease course. Conversely, using anti-IL-22-neutralizing antibody with Ficz cancels the Ficz-dependent protection.

Paradoxically, IL-22 was also shown to contribute to the pathogenesis of chronic IBD in mice by the mechanism similar to its pathogenic role in other disease models. IL-10<sup>-/-</sup> CD45RB<sup>low</sup> CD25<sup>+</sup> CD4<sup>+</sup> memory T cells can transfer colitis to Rag<sup>-/-</sup> hosts. Unlike naive T-cell colitis, this model predominantly garners Th17 responses in the intestines and transfer of IL-22–/– memory T cell alleviates the disease. In this model, colitis is characterized by hyperplasia, thickened mucosa, and interestingly heightened IFN-γ response. Pathogenic role of IL-22 of CD4<sup>+</sup> T-cell origin was also demonstrated in a pathogen-induced ileitis model. Toxoplasma gondii infection of B6 mice results in CD4<sup>+</sup> T cell–driven ileitis, dependent on IFN-γ and TNF-α, and death. Pathogenic role of IL-22 of CD4<sup>+</sup> T-cell origin was also demonstrated in a pathogen-induced ileitis model. T cell drive production of IL-17A.

**ILC3, γδ T, AND NKT CELLS IN IL-23R–MEDIATED PATHOLOGY**

**Group 3 ILCs**

The major innate cell population that expresses IL-23R in the intestines is group 3 ILC, which is composed of LTi cells and ILC3s. Various ILC3s were described in humans and mice based on the effector cytokines they produce. These include, but are not limited to, (1) IL-22–producing NCR<sup>+</sup> ILC3, which are also called ILC22, NK22, NKR-LTi, or NCR22<sup>+</sup>; (2) NCR<sup>-</sup> IL-17A<sup>-</sup> IFN-γ<sup>+</sup> double-producing ILC3<sup>+</sup>; and finally, (3) NCR<sup>-</sup> IL-17A<sup>+</sup> ILC3s. All of these subsets of ILC3s respond to IL-23 and produce the aforementioned cytokines; however, it is not yet clear whether they are the same cells expressing different set of genes in response to distinct stimuli or specialized set of cells differentiated a priori. More recently, lineage studies in mice put these subsets into perspective. It was proposed that murine gut is first colonized by CCR6<sup>+</sup>T<sup>+</sup>RORγ<sup>+</sup> ILC3s during fetal development, and these cells resemble to LTi cells and produce IL-17A, IL-17F, and IL-22. Additionally, a CCR6<sup>+</sup>T<sup>+</sup>RORγ<sup>+</sup> subset appears after birth whose development is dependent on AhR and microbiota. This latter CCR6<sup>+</sup>T<sup>+</sup>RORγ<sup>+</sup> subset can upregulate T-bet expression in a IL-23–dependent and microbiota-dependent manner and gives rise to CCR6<sup>-</sup>Nkpg4<sup>+</sup>RORγ<sup>+</sup> subset (known as NK22 cells and do not express IL-17). NK22 cells in turn can give rise to the CCR6<sup>-</sup>Nkpg4<sup>+</sup>RORγ<sup>+</sup> cells (ILC1) in certain milieu. However, other data argues that fetal LTi cells do not give rise to NK22 or other RORγ<sup>+</sup> ILCs. Nevertheless, data indicate that ILC3 are rapidly tunable cells. It is likely that in appropriate conditions, even NK22 cells may produce IL-17 cytokine. Supporting this, resting human tonsil ILC3s (c-kit+Nkpg4<sup>+</sup>) express IL-22, but not IL-17A without stimulation, but with extended phosphor 12-myratiste 13-acetyl-ionoycin stimulation start producing IL-17A. Additionally, some of the ILC3s were identified in different models of colitis. NCR<sup>-</sup> IL-17A<sup>-</sup> IFN-γ<sup>-</sup> double-producing ILC3s were found during H. hepaticus infection; NCR<sup>-</sup> IL-17A<sup>-</sup> expressing ILC3s (with marginal IL-22 production) were described in Truc21<sup>+</sup>Rag2<sup>-/-</sup> (TRUC) mice, which were shown to develop H. typhlonius–driven colitis. Regardless of the questions concerning ontology, within the past few years, more studies demonstrated that these IL-23R–responsive innate cells are involved in the mouse models of colitis and may also be instrumental in the human IBD. In fact, Geremia et al. documented elevated expression of IL-22, IL-26, IL-17A, and IL-17F in intestinal tissue of both CD and UC patients compared with the healthy controls. They showed that both T and non-T cells were the source of these Th17-type cytokines, implicating an active IL-23/IL-23R signaling pathway in both adaptive and innate immune cells in the etiology of IBD. In addition to CD3<sup>+</sup> cells, IL-17A was highly upregulated in the ILC of IBD patients compared with the controls, suggesting a role for ILCs in the IBD pathogenesis. Of note, they reported an increased frequency of IL-17–producing ILCs in the CD but not UC patients. Furthermore, they also observed an increase in IL26, Rorc, Ahr, and Il23r expression in the mucosal homogenates of IBD patients. Finally, NK cell marker CD56<sup>+</sup> ILCs were the major source of IL-22, and CD56<sup>-</sup> ILCs were the major source of IL-17A.

Mouse studies proved very useful for dissecting the role of ILC3-derived Th17 cytokines in innate colitis. Buonocore et al., demonstrated in a landmark article that in a previously established H. hepaticus–driven innate colitis model, 14,20 which is dependent on IL-23, IFN-γ<sup>+</sup> IL-17<sup>-</sup> producing ILC3s were the major driver of colitis; as such, deletion of ILC3 by crossing Rorc<sup>-/-</sup> mice to a Rag<sup>-/-</sup> background or depletion through anti-Thy1 antibodies.
ameliorated innate colitis. Similarly, neutralization of IL-17A or IFN-γ alone or together had the same effect. Another innate colitis model that was previously described by Uhlig et al., which utilized the injection of anti-CD40 antibodies, was also explored by the same investigators. In this model, colitis was also shown to be IL-23 dependent and deletion of Rorc gene, depletion of Thy1+ cells, or neutralization of IFN-γ resulted in improved disease progression if not a complete recovery, implicating this model of IFN-γ−/− ILC3s in disease pathogenesis. Studies from Diefenbach’s laboratory revealed that RORγt+NKp46− ILC3s (NCR− ILC3) can upregulate NKp46 in vivo, giving rise to NCR+ ILC3 and subsequently downregulate their RORγt in the presence of IL-12 or IL-15 and assume a Th1-like or NK-like phenotype and named these cells as RORγt−NKR-LT( currently called ILC1). These cells were shown to produce IFN-γ and considered as the major driver of IFN-γ−dependent colitis in the anti-CD40-induced colitis model by means of elegant cell transfer experiments. More recently, this phenomenon was recapitulated in humans. Human ILC3 characterized as CD3−CD127−c-kit+ NKp44+ can downregulate RORγt and IL-23R when cultured in the presence of IL-12 and IL-2 upregulate T-bet, and then produce IFN-γ. These cells are categorized as non-NK ILC1. Interestingly, although human ILC1 express TBX21, IFN-γ, and CXCR3 and have a Th1 effector phenotype, there is residual IL-23R and RORC expression in these cells. Indeed, at least ex vivo when cultured in the presence of IL-23 and IL-2, they downregulate T-bet, although they are unable to revert to an ILC3 phenotype. It is yet to be studied whether IL-23R signaling by itself or in synergy with factors in ILC1 bears functional consequences in vivo. Additionally, ILC1 percentage in CD-inflamed intestine was shown to be elevated and in humanized mice treated with DSS; however, causality with the disease is also unclear.

A pathogenic role for IL-17A cytokine of ILC3 origin also came from studies with Tbx21−/−/Rag2−/− (TRUC) mice. TRUC mice develop spontaneous IBD in a microbe-independent fashion. Interestingly, the disease is TNF-α dependent until mice are 12-week old after which blockade of TNF-α is ineffective. Recently, it was shown that colitis in these mice is mediated by ILC3 and reduced by IL-23 or IL-17A neutralization or IL-7R blockade. Interestingly, TNF-α produced by CD11b+CD103− DCs synergized with IL-23 mediated IL-17A production by ILC3s, and Tbx21 expression in ILC3s was refractory to IL-17A expression shown by T-bet binding to IL-17A locus.

Both pathogenic and protective roles for ILC3-derived IL-22 have been reported. Deletion of ILC3s by crossing Rag2−/− mice to Rorc−/− not only renders double-knockout mice more susceptible to DSS-induced colitis but also delays the recovery. The repair of epithelial damage induced by DSS is mediated by IL-22. In agreement with this, the use of IL-22-deficient immunocompetent mice or targeting upstream IL-22-inducing signaling axis component IL-23R on a Rag2−/− mice results in more destruction to the intestines in response to DSS challenge, which could be corrected by recombinant IL-22-Fc injections. It must be noted that dosing of IL-22 in such experiments is critical. Data argue that high doses or sustained IL-22 expression does not provide protection in this model (Amgen symposium). It is perplexing that in the presence of T cells, ablation of IL-23R or IL-23p19 does not exacerbate but conversely ameliorates DSS-induced colitis. It is likely that in T cell–sufficient and B cell–sufficient mice, IL-23R/IL-17F axis or other Th17 cell–derived pathogenic cytokines may contribute to colitogenesis; thus, when upstream IL-23R or IL-23p19 is removed from the system, even though the repair mechanism through IL-22 is unavailable, pathology is limited. Besides the cell-proliferative effect of IL-22, very recent studies suggested that by restricting growth of certain genera of bacteria in the steady-state IL-22 may contribute to protection from IBD. Zenewicz et al. showed that IL-22−/− mice intestine differs in representation of 14 different genera compared with WT mice, and cohousing of WT with KO mice is sufficient to transfer the susceptibility to DSS-induced colitis. Another study using AhR−/− Rorc−/− mice showed that reduced IL-22 levels in the murine intestine also results in overgrowth of SFB, which in turn promotes Th17 differentiation, thereby rendering mice more prone to Th17–driven adaptive and spontaneous colitis. Importantly, gut IL-22 is mainly of innate cell origin, and these examples show different aspects of IL-22–mediated protection from IBD.

In addition to the regenerative/protective role of IL-22, our studies with the anti-CD40–driven innate colitis model revealed a pathogenetic role for IL-23R/IL-22 axis in ILC3. By neutralizing IL-22 in Rag2−/− mice or restoring IL-22 expression in IL-23R−/−Rag2−/− animals, we demonstrated that IL-23 may drive colitis partly through IL-22 by modulating IL-10, IFN-γ levels, and neutrophil recruitment. This is not surprising given that IL-22 may have pathogenic or protective properties even in the adaptive colitis models, induced by CD45RBlow memory and CD45RAbright naive T cells, respectively.

γδ T Cells

γδ T cells are abundantly found in the intestine and constitute up to 30% of intraepithelial lymphocytes. The majority of γδ T cells in the IEL is CD8β+ and develops extrathymically in the cryptopatches. Increased percentage and absolute numbers of γδ T cells have been reported in the peripheral blood of active IBD patients and in the intestines. During DSS-induced colitis, γδ T cells have been shown to localize around the lesions. Tcrδ−/− mice was shown to be more prone to DSS-induced colitis associated with reduced regeneration and epithelial tissue repair, which correlates with a reduction in epithelial cell mitogen keratinocyte growth factor secreted by γδ T cells. This protective effect was supported by others as well. In rats, depletion of γδ T cells has also been reported to exacerbate TNBS-induced colitis. More recently, the protective effect of γδ T cells in DSS-induced colitis was shown to be mediated through enhanced IL-22 secretion. This protective effect is enhanced by retinoic acid and occurs through retinoic acid receptor binding of Il17 promoters, which results in enhanced production of IL-22 when γδ T cells stimulated through IL-23 combined with IL-18.

On the other hand, colitis-aggravating roles for γδ T cells were also documented. Tcrα−/− mice develop UC-like spontaneous
colitis in a microbiota-dependent fashion, and deletion of γδ T cells in these mice ameliorated the colitis pathology. Similar to DSS-induced colitis in Tcrδ−/− mice; Tcrα−/−γ−/− double knockouts had reduced neutrophil infiltration, yet its perplexing that this reduction resulted in exacerbated disease in the former but alleviated the spontaneous colitis induced by Tcrα deficiency. Using CD4+ T-cell transfer, Do et al. demonstrated a resistance to colitis in Tcrδ−/− mice compared with Tcrδ+/− mice, which were restored by the cotransfer of IL-17-producing CCR6+ but not CCR6− γδ T cells with naive T cells, implicating them as proinflammatory. Accordingly, the presence of γδ T cells augmented IL-17+ Th17 cells and IFN-γ+ Th1 cells.

Finally, Park et al. recently showed in a spontaneous colitis model where phosphoinositide-dependent protein kinase 1 (Pdk1) is deleted in CD4+ cells that TCRγδ+ cells were responsible for colitis, and they required intestinal symbionts for their pathogenicity. However, studies directly assessing the role of IL-23R in γδ T cells are lacking.

**NKT Cells and Other Innate IL-23R+ Cells in IBD**

Type 1 NKT (iNKT) cells have been shown to express RORγt and IL-23R and produce IL-17. These cells respond to IL-23 and can produce large amounts of IL-22 and IL-17 when costimulated with IL-23 and IL1β. Type 1 NKT cells have been reported to be reduced in the blood and intestinal tissue of CD and UC patients. The role of these cells in IBD has been reviewed (see review by Liao et al.). Owing to the IL-4-producing and IL-13–producing and Th2 response–promoting abilities of these cells, they have been described as protective cells in various murine IBD models. In DSS-induced TNBS-induced, naive CD4+ T-cell transfer–induced, and T gondii–induced models of colitis, these cells were shown to play a protective role. However, the contribution of IL-22 or IL-17 to this protection has not yet been investigated.

More recently, a study from Casey Weaver’s Lab demonstrated that sorted neutrophils can also produce IL-22 and IL-17 in response to IL-23 stimulation. During DSS-induced colitis, this contribution becomes significant; as such, depletion of neutrophils through anti-Gr-1 antibodies exacerbates colitis and delays the recovery.

**FUNCTIONAL CONSEQUENCES OF SINGLE-NUCLEOTIDE POLYMORPHISMS IN THE IL-23R–SIGNALING PATHWAY**

Various single-nucleotide polymorphisms (SNP) in the IL-23R locus have been identified as susceptibility or resistance factors for IBD. Some of these SNPs were recently studied in detail. rs11209026 or R381Q SNP was identified as a protective variant for CD and UC. Arg-381 is located in the cytoplasmic domain of IL-23R protein and is highly conserved among species. However, Gln-381 allele is rarer than Arg and was reported to provide protection from CD in Jewish and non-Jewish cohorts. A subsequent study demonstrated that CD8+ and memory CD4+ T cells purified from Gln-381 IL-23R allele carriers showed less IL-17 and IL-22 production in response to IL-23 stimulation, and R381Q carriers had fewer circulating Th17 and Tc17 cells compared with healthy individuals who lack R381Q SNP. Furthermore, R381Q IL-23R transfected cell lines displayed reduced STAT3 phosphorylation compared with control IL-23R. Loss of IL-23R function in response to IL-23 stimulation by Th17 cells in individuals carrying R381Q allele was confirmed independently by another study, although differentiation of Th17 cells from naive CD4+ T cells reported to be unaltered. In addition to cytokine stimulation, peripheral blood mononuclear cells from individuals with R381Q variant produce less IL-17 in response to *Borrelia burgdorferi*, a potent inducer of Th17 responses. These reports argue that R381Q SNP in IL-23R is a loss of function mutation and may provide a mechanistic explanation for resistance to CD and UC.

p.Arg86Gln, p.Gly149Arg, and p.Val362Ile variants of IL-23R were also found to be protective against CD. The latter two protect against UC as well. p.Gly149Arg affects a highly conserved extracellular domain of IL-23R, whereas p.Arg86Gln and p.Val362Ile seem to be variants in poorly conserved domains. Joint contribution of these 3 rare variants to the protection against CD is lower than that of R381Q allele (0.44% versus 1.23%). Although these SNPs are believed to decrease IL-23R activity, experimentally, this has yet to be demonstrated.

Other IL-23R SNPs have been discovered as risk factors for CD development. rs10889677 is a transversion in the end result is increased IL-23R messenger RNA and protein expression. Although from a very simplistic perspective, aforementioned mechanistic SNP studies suggest that susceptibility polymorphisms of IL-23R may be gain of function mutations and risk alleles may be loss of function mutations, data from various animal models show that different effector cytokines produced as end products of IL-23R signaling can cause or but also protect from IBD in different contexts. It is likely that some SNPs may preferentially favor or impair a downstream Th17 cytokine axis while not affecting others. Additionally, these SNPs may impact various IL-23–responding innate and adaptive cells described in the previous sections differentially. Future animal models harboring equivalent of these human SNPs will be invaluable to understand the mechanisms by which the IBD pathogenesis occurs.

**CONCLUSIONS**

After the discovery of IL-23 and the subsequent identification of Th17 cells, studies with various murine models of...
experimental IBD helped the recognition of the essential role of IL-23/IL-23R signaling in chronic gut inflammation. In addition to adaptive Th17 cells, group 3 ILCs have emerged as the potential mediator of pathogenesis. We are still far from understanding how IL-23R signaling in each of these different cell subsets is contributing to pathology. Further complexity arise given these different groups of cells are also heterogeneous and there is plasticity among them. Moreover, IL-23 stimulation turns on the expression of a number of cytokines by target cells, and those cytokines act in a context-dependent manner, and it seems that they play opposite roles in different experimental models. Although these findings are very useful for tailoring the therapeutics, and rethinking potential side effects, they show the complexity of the disease pathogenesis. Understanding which aspect, stage, or type of CD each murine model may reflect is essential.

Antibodies targeting IL-23R signaling components are under trial. Anti-p40 (Ustekinumab) trials were able to generate some clinical response; however, no effect on remission has been observed. However, anti–IL-17A antibodies exacerbated the disease in clinical trials. We will probably see supplemental IL-22 injection trials owing to its role in epithelial regeneration; however, this cytokine can have inflammatory properties, as described side effects also might be expected. It is also conceivable in the near future that we will also see human studies that will target multiple cytokines at once.

**REFERENCES**

1. Oppermann B, Lesley R, Blom B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity*. 2000;13:715–725.
2. Becker C, Wirtz S, Blessing M, et al. Constitutive p40 promoter activation and IL-23 production in the terminal ileum mediated by dendritic cells. *J Clin Invest.* 2003;112:693–706.
3. Gerosa F, Baldani-Guerra B, Lyakh LA, et al. Differential regulation of interleukin 12 and interleukin 23 production in human dendritic cells. *J Exp Med.* 2008;205:1447–1461.
4. Mills KH. Induction, function and regulation of IL-17-producing T cells. *Eur J Immunol.* 2008;38:2636–2649.
5. Carmody RJ, Ruan Q, Liou HC, et al. Essential roles of c-Rel in TLR-4–induced IL-23 p19 gene expression in dendritic cells. *J Immunol.* 2007;178:186–191.
6. Kom T, Bettelli E, Oukka M, et al. IL-17 and Th17 cells. *Anna Rev Immunol.* 2009;27:485–517.
7. Goryel S, Neurath MF, Goldman M. How microorganisms tip the balance between interleukin-12 family members. *Nat Rev Immunol.* 2008;8:81–86.
8. Varol C, Vallon-Eberhard A, Elinav E, et al. Intestinal lamina propria dendritic cell subsets have different origin and functions. *Immunity*. 2009;31:502–512.
9. Siddiqui KR, Laffont S, Powirz F. E-cadherin marks a subset of inflammatory dendritic cells that promote T cell-mediated colitis. *Immunity*. 2010;32:557–567.
10. Kinnebrew MA, Buffie CG, Diehl GE, et al. interleukin 23 production by intestinal CD103(+)/CD11b(+) dendritic cells in response to bacterial flagellin enhances mucosal innate immune defense. *Immunity*. 2012;36:276–287.
11. Satpathy AT, Briseno CG, Lee JS, et al. Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens. *Nat Immunol.* 2013;14:937–948.
12. Uhlig HH, McKenzie BS, Hue S, et al. Differential activity of IL-12 and IL-23 in mucosal and systemic innate immune pathology. *Immunity*. 2006;25:309–318.
13. Yen D, Cheung J, Scheeren H, et al. IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. *J Clin Invest.* 2006;116:1310–1316.
14. Kullberg MC, Jankovic D, Feng CG, et al. IL-23 plays a key role in Helicobacter hepaticus-induced T cell-dependent colitis. *J Exp Med.* 2006;203:2485–2494.
15. Kamada N, Hisamatsu T, Okamoto S, et al. Unique CD14 intestinal macrophages contribute to the pathogenesis of Crohn disease via IL-23/IFN-gamma axis. *J Clin Invest.* 2008;118:2269–2280.
16. Liu Z, Yadav PK, Xu X, et al. The increased expression of IL-23 in inflammatory bowel disease promotes intraepithelial and lamina propria lymphocyte inflammatory responses and cytotoxicity. *J Leukoc Biol.* 2011;89:597–606.
17. Holta V, Klemetti P, Sipponen T, et al. IL-23/IL-17 immunity as a hallmark of Crohn’s disease. *Inflamm Bowel Dis.* 2008;14:1175–1184.
18. Abe K, Nguyen KP, Fine SD, et al. Conventional dendritic cells regulate the outcome of colonic inflammation independently of T cells. *Proc Natl Acad Sci U S A.* 2007;104:17022–17027.
19. Wiekowski MT, Leach MW, Evans EW, et al. Ubiquitous transgenic expression of the IL-23 subunit p19 induces multiorgan inflammation, running, infertility, and premature death. *J Immunol.* 2001;166:7563–7570.
20. Hue S, Ahern P, Buonocore S, et al. Interleukin-23 drives innate and T cell-mediated intestinal inflammation. *J Exp Med.* 2006;203:2473–2483.
21. Cox JH, Kjøvin NM, Ota N, et al. Opposing consequences of IL-23 signaling mediated by innate and adaptive cells in chemically induced colitis in mice. *Mucosal Immunol.* 2012;5:99–109.
22. Wirtz S, Finotto S, Kanzler S, et al. Cutting edge: chronic intestinal inflammation in STAT-4 transgenic mice: characterization of disease and adoptive transfer by TNF– plus IFN-gamma-producing CD4+ T cells that respond to bacterial antigens. *J Immunol.* 1999;162:1884–1888.
23. Becker C, Domhoff H, Neufert C, et al. Cutting edge: IL-23 crosses-regulates IL-12 production in T cell-dependent experimental colitis. *J Immunol.* 2006;177:2760–2764.
24. Parham C, Cirriha M, Timans J, et al. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12beta1 and a novel cytokine receptor subunit. *J Immunol.* 2002;168:5699–5708.
25. Cho ML, Kang JW, Moon YM, et al. STAT3 and NF-kappaB signaling mediated by innate and adaptive cells in chemically induced colitis. *Mucosal Immunol.* 2012;5:99–109.
26. Floss DM, Mrotzek S, Klocker T, et al. Identification of canonical tyrosine-dependent and non-canonical tyrosine-independent STAT3 activation sites in the intracellular domain of the interleukin 23 receptor. *J Biol Chem.* 2013;288:19386–19400.
27. Zindl CL, Lai JF, Lee YK, et al. IL-22-producing neutrophils contribute to antimicrobial defense and restitution of colonic epithelial integrity during colitis. *Proc Natl Acad Sci U S A.* 2013;110:12768–12773.
28. Awasthi A, Riel-Blanco L, Jager A, et al. Cutting edge: IL-23 receptor gfp reporter mice reveal distinct populations of IL-17-producing cells. *J Immunol.* 2009;182:5904–5908.
29. Rachitskaya AV, Hansen M, Horai R, et al. Cutting edge: NK cells constitutively express IL-23 receptor and ROGammat and rapidly produce IL-17 upon receptor ligation in an IL-6–independent fashion. *J Immunol.* 2008;180:5167–5171.
30. Zhou L, Ivanov II, Sopelko R, et al. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol.* 2007;8:967–974.
31. Ghoreschi K, Laurence A, Yang XP, et al. Generation of pathogenic T(H)17 cells in the absence of TGF-beta signalling. *Nature.* 2010;467:967–971.
32. Coccia M, Harrison OJ, Schiering C, et al. IL-1beta mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4+ T cells. *J Exp Med.* 2012;209:1595–1609.
33. Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature.* 2006;441:235–238.
34. Coccia M, Harrison OJ, Schiering C, et al. IL-1beta mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting
innate lymphoid cells and CD4+ Th17 cells. *J Exp Med.* 2012;209:1595–1609.

35. Izcue A, Hue S, Buonocore S, et al. Interleukin-23 restrains regulatory T cell activity to drive T cell-dependent colitis. *Immunity.* 2008;28:559–570.

36. Ahern PP, Schiering C, Buonocore S, et al. Interleukin-23 drives intestinal inflammation through direct activity on T cells. *Immunity.* 2010;33:279–288.

37. Osorio F, LeibundGut-Landmann S, Lochner M, et al. DC activated via dectin-1 convert Treg into IL-17 producers. *Eur J Immunol.* 2008;38:3274–3281.

38. Eken A, Singh AK, Treuting PM, et al. IL-23R(+)/innate lymphoid cells induce colitis via interleukin-22-dependent mechanism. *Mucosal Immunol.* 2014;7:143–154.

39. Kobayashi T, Okamoto S, Hisamatsu T, et al. IL-23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn’s disease. *Gut.* 2008;57:1682–1689.

40. Duerr RH, Taylor KD, Brant SR, et al. A genome-wide association study identifies IL-22R as an inflammatory bowel disease gene. *Science.* 2006;314:1461–1463.

41. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature.* 2007;447:661–678.

42. Ivanov II, Atarashi K, Mannel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell.* 2009;139:485–498.

43. Qiu J, Guo X, Chen ZM, et al. Group 3 innate lymphoid cells inhibit T-cell-mediated intestinal inflammation through aryl hydrocarbon receptor signaling and regulation of microflora. *Immunity.* 2013;39:386–399.

44. Brain O, Owens BM, Pichulik T, et al. The intracellular sensor NOD2 induces microRNA-29 expression in human dendritic cells to limit IL-23 release. *Immunity.* 2013;39:521–536.

45. Cuthbert AP, Fisher SA, Mirza MM, et al. The contribution of NOD2 gene mutations to the risk and site of disease in inflammatory bowel disease. *Gastroenterology.* 2002;122:867–874.

46. Fossiez F, Djossou O, Chomarat P, et al. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J Exp Med.* 1996;183:2593–2603.

47. Laan M, Cui ZH, Hoshino H, et al. Neutrophil recruitment by segmented filamentous bacteria and CD4+ Th17 cells induce murine chronic intestinal inflammation. *J Exp Med.* 2006;205:1063–1075.

48. Sugimoto K, Ogawa A, Mizoguchi E, et al. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. *J Clin Invest.* 2008;118:534–544.

49. Pickert G, Neufert C, Leppkes M, et al. STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. *J Exp Med.* 2009;206:1465–1472.

50. Zheng Y, Danilenko DM, Valdez P, et al. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity.* 2008;29:947–957.

51. Monteleone I, Rizzo A, Sarra M, et al. Aryl hydrocarbon receptor-induced signals up-regulate IL-22 production and inhibit inflammation in the gastrointestinal tract. *Gastroenterology.* 2011;141:237–248, e231.

52. Zheng Y, Danilenko DM, Valdez P, et al. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature.* 2007;445:648–651.

53. Kamakura M, Huber S, Zenevitz LA, et al. Memory/effector (CD45RB (lo)) CD4 T cells are controlled directly by IL-10 and cause IL-22-dependent intestinal pathology. *J Exp Med.* 2011;208:1027–1040.

54. Khan IA, Schwartzman JD, Matsuura T, et al. A dichotomous role for nitric oxide during acute Toxoplasma gondii infection in mice. *Proc Natl Acad Sci U S A.* 1997;94:13955–13960.

55. Monteleone I, Rizzo A, Sarra M, et al. Lamina propria CD4+ T lymphocytes synergize with murine intestinal epithelial cells to enhance proinflammatory response against an intracellular pathogen. *J Immunol.* 2002;168:2988–2996.

56. Munoz M, Heimesaat MM, Danker K, et al. Interleukin (IL-23) mediates Toxoplasma gondii-induced immunopathology in the gut via matrixmetalloproteinase-2 and IL-22 but independent of IL-17. *J Exp Med.* 2009;206:3047–3059.

57. Spits H, Arts D, Colonna M, et al. Innate lymphoid cells—a proposal for uniform nomenclature. *Nat Rev Immunol.* 2013;13:145–149.

58. Cella M, Fuchs A, Vermi W, et al. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature.* 2009;457:722–725.

59. Sanos SL, Bui VL, Mortha A, et al. RORγt and commensal microflora are required for the differentiation of mucosal interleukin-22-producing NKP46+ cells. *Nat Immunol.* 2009;10:83–91.

60. Buonocore S, Ahern PP, Uhlig HH, et al. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature.* 2010;464:1371–1375.

61. Powell N, Walker AW, Stolarczyk E, et al. The transcription factor T-bet regulates intestinal inflammation mediated by interleukin-7 receptor+ innate lymphoid cells. *Immunity.* 2012;37:674–684.

62. Klose CS, Kiss EA, Schierzveck V, et al. A T-bet gradient controls the fate and function of CCR6-RORgammat+ innate lymphoid cells. *Nature.* 2013;494:261–265.

63. Sawa S, Cherrier M, Lochner M, et al. Lineage relationship analysis of RORgammat+ innate lymphoid cells. *Science.* 2010;330:665–669.
83. Bernink JH, Peters CP, Munneke M, et al. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. *Nat Immunol.* 2013;14:221–229.

84. Geremia A, Arancibia-Carcamo CV, Fleming MP, et al. IL-23-responsive innate lymphoid cells are increased in inflammatory bowel disease. *J Exp Med.* 2011;208:1127–1133.

85. Vonarbourg C, Mortha A, Bui VL, et al. Regulated expression of nuclear receptor RORgammaT confers distinct functional fates to NK cell receptor-expressing RORgamma(+) innate lymphocytes. *Immunity.* 2010;33:736–751.

86. Takayama T, Kamada N, Chinen H, et al. Imbalance of NKp44(+) NKp46(−) and NKp44(−)NKp46(+) natural killer cells in the intestinal mucosa of patients with Crohn’s disease. *Gastroenterology.* 2010;139:882–892, 892 e881–883.

87. Garrett WS, Lord GM, Punit S, et al. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell.* 2007;131:33–45.

88. Sawaya S, Lochner M, Satoh-Takada Y, et al. RORgamma(+) innate lymphoid cells regulate intestinal homeostasis by integrating negative signals from the symbiotic microbiota. *Nat Immunol.* 2011;12:320–326.

89. Kimura K, Kanai T, Hayashi A, et al. Dysregulated balance of retinoic acid-related orphan receptor gammaH-dependent innate lymphoid cells is involved in the pathogenesis of chronic DSS-induced colitis. *Biochem Biophys Res Commun.* 2012;427:694–700.

90. Zenewicz LA, Yin X, Wang G, et al. IL-22 deficiency alters colonic microbiota to be transmissible and colitogenic. *J Immunol.* 2013;190:5306–5312.

91. Zenewicz LA, Flavell RA. IL-22 and inflammation: leukin’ through a glass onion. *Eur J Immunol.* 2008;38:3265–3268.

92. Hayday A, Trigell R. Immunoregulation in the tissues by gammadelta T cells. *J Exp Med.* 2011;208:1127–1133.

93. Grose RH, Thompson FM, Baxter AG, et al. Deficiency of invariant NK T cells in Crohn’s disease and ulcerative colitis. *Dig Dis Sci.* 2007;52:1415–1422.

94. McVay LD, Li B, Biancaniello R, et al. Changes in human mucosal T regulatory cells maintain intestinal homeostasis by suppressing gammadelta T cells. *J Immunol.* 2010;186:4546–4550.

95. Park SG, Mathur R, Long M, et al. T regulatory cells maintain intestinal homeostasis by suppressing gammadelta T cells. *Immunol.* 2010;33:791–803.

96. Doisne JM, Becourt C, Amniai L, et al. Skin and peripheral lymph node invariant NKT cells are mainly retinoic acid receptor-related orphan receptor (gamma)a(+) and respond preferentially under inflammatory conditions. *J Immunol.* 2009;183:2142–2149.

97. Doisne JM, Soulard V, Becourt C, et al. Cutting edge: crucial role of IL-1 and IL-23 in the innate IL-17 response of peripheral lymph node NK1.1- invariant NKT cells to bacteria. *J Immunol.* 2011;186:662–666.

98. Grose RH, Thompson FM, Baxter AG, et al. Deficiency of invariant NK T cells in Crohn’s disease and ulcerative colitis. *Dig Dis Sci.* 2007;52:1415–1422.

99. Liao CM, Zimmer MI, Wang CR. The functions of type I and type II natural killer T cells in inflammatory bowel diseases. *Inflamm Bowel Dis.* 2013;19:1330–1338.

100. Saubermann LJ, Beck P, De Jong YP, et al. Activation of natural killer T cells by alpha-galactosylceramide in the presence of CD1d provides protection against colitis in mice. *Gastroenterology.* 2000;119:119–128.

101. Ueno Y, Tanaka S, Sumii M, et al. Single dose of OCH improves mucosal T helper type 1/T helper type 2 cytokine balance and prevents experimental colitis in the presence of valpha14 natural killer T cells in mice. *Inflamm Bowel Dis.* 2005;11:35–41.

102. Shiholet O, Kalish Y, Klein A, et al. Adoptive transfer of ex vivo immune-programmed NKT lymphocytes alleviates immune-mediated colitis. *J Leukoc Biol.* 2004;75:76–86.

103. Hornung M, Farkas SA, Sattler C, et al. DXS(+) NKT cells induce the death of colitis-associated cells: involvement of programmed death ligand-1. *Eur J Immunol.* 2006;36:1210–1221.

104. Smiley ST, Lanthier PA, Couper KN, et al. Exacerbated susceptibility to infection-stimulated immunopathology in CD1d-deficient mice. *J Immunol.* 2005;174:7904–7911.

105. Ronet C, Darche S, Litte de Moraes M, et al. NKT cells are critical for the initiation of an inflammatory bowel response against Toxoplasma gondii. *J Immunol.* 2005;175:899–908.

106. McGovern DP, Taylor KD, Landers C, et al. MAGI2 genetic variation and inflammatory bowel disease. *Inflamm Bowel Dis.* 2009;15:75–83.

107. Sarin R, Wu X, Abraham C. Inflammatory disease protective R381Q IL23 receptor polymorphism results in decreased primary CD4+ and CD8+ human T-cell functional responses. *Proc Natl Acad Sci U S A.* 2011;108:9560–9565.

108. Di Meglio P, Di Cesare A, Laggner U, et al. The IL23R R381Q gene variant protects against immune-mediated diseases by impairing IL-23-induced Th17 effector response in humans. *PLoS One.* 2011;6:e217160.

109. Pidaseva S, Trifari S, Phillips A, et al. Functional studies on the IBD susceptibility gene IL23R implicated reduced receptor function in the protective genetic variant R381Q. *PLoS One.* 2011;6:e25038.

110. Oosting M, ter Hofstede H, van de Pouw Kraan TC, et al. Cutting edge: a variant in IL23R coding variants protecting against inflammatory bowel disease. *Nat Genet.* 2011;43:43–47.

111. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc.* 2009;4:1073–1081.

112. Di Meglio P, Di Cesare A, Laggner U, et al. The IL23R R381Q gene variant protects against immune-mediated diseases by impairing IL-23-induced Th17 effector response in humans. *PLoS One.* 2011;6:e217160.

113. Pidaseva S, Trifari S, Phillips A, et al. Functional studies on the IBD susceptibility gene IL23R implicated reduced receptor function in the protective genetic variant R381Q. *PLoS One.* 2011;6:e25038.

114. Oosting M, ter Hofstede H, van de Veerdonk FL, et al. Role of interleukin-12–IL-17 receptor signaling for IL-17 responses in human Lyme disease. *Infect Immun.* 2011;79:4681–4687.

115. Momozawa Y, Mni M, Nakamura K, et al. Resequencing of positional candidates identifies low frequency IL23R coding variants protecting against inflammatory bowel disease. *Nat Genet.* 2011;43:43–47.

116. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc.* 2009;4:1073–1081.

117. Zwiers A, Kraal L, van de Pouw Kraan TC, et al. Cutting edge: a variant of the IL-23R gene associated with inflammatory bowel disease induces loss of microRNA regulation and enhanced protein production. *J Immunol.* 2012;188:1573–1577.

118. Franke A, McGovern DP, Barrett JC, et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn’s disease susceptibility loci. *Nat Genet.* 2010;42:1118–1125.

119. Barrett JC, Hansoul S, Nicolae DL, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn’s disease. *Nat Genet.* 2008;40:955–962.

120. Fuss U, Becker C, Yang Z, et al. Both IL-12p70 and IL-23 are synthesized during active Crohn’s disease and are down-regulated by treatment with anti-IL-12 p40 monoclonal antibody. *Inflamm Bowel Dis.* 2006;12:9–15.

121. Mannon PJ, Fuss UJ, Mayer L, et al. Anti-interleukin-12 antibody for active Crohn’s disease. *N Engl J Med.* 2004;351:2099–2079.

122. Sandborn WJ, Feagan BG, Fedorak RN, et al. A randomized trial of Ustekinumab, a human interleukin-12-23 monoclonal antibody, in patients with moderate-to-severe Crohn’s disease. *Gastroenterology.* 2008;135:1130–1140.