

Interleukin-23 in the Pathogenesis of Inflammatory Bowel Disease and Implications for Therapeutic Intervention

Gavin W. Sewell,^{a,b} Arthur Kaser^{a,b}

^aCambridge Institute of Therapeutic Immunology and Infectious Disease, Jeffrey Cheah Biomedical Centre, University of Cambridge, Cambridge, UK

^bDivision of Gastroenterology and Hepatology, Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK

Corresponding author: Arthur Kaser, Cambridge Institute of Therapeutic Immunology and Infectious Disease (CITIID), Jeffrey Cheah Biomedical Centre, Cambridge Biomedical Campus, University of Cambridge, Puddicombe Way, Cambridge CB2 0AW, UK. Tel: +44 1223 331130; Email: ak729@cam.ac.uk

Abstract

The interleukin-23 [IL-23] cytokine, derived predominantly from macrophages and dendritic cells in response to microbial stimulation, has emerged as a critical promoter of chronic intestinal inflammation. Genome-wide association studies linking variants in *IL23R* to disease protection, bolstered by experimental evidence from colitis models, and the successful application of therapies against the IL-12/IL-23 shared p40 subunit in the treatment of inflammatory bowel disease [IBD] all provide compelling evidence of a crucial role for IL-23 in disease pathogenesis. Moreover, targeting the p19 subunit specific for IL-23 has shown considerable promise in recent phase 2 studies in IBD. The relative importance of the diverse immunological pathways downstream of IL-23 in propagating mucosal inflammation in the gut, however, remains contentious. Here we review current understanding of IL-23 biology and explore its pleiotropic effects on T cells, and innate lymphoid, myeloid and intestinal epithelial cells in the context of the pathogenesis of IBD. We furthermore discuss these pathways in the light of recent evidence from clinical trials and indicate emerging targets amenable to therapeutic intervention and translation into clinical practice.

Key words: Interleukin-23; Crohn's disease; ulcerative colitis

1. Introduction

Crohn's disease [CD] and ulcerative colitis [UC] represent the two main clinical forms of chronic inflammatory bowel disease [IBD].^{1,2} Susceptibility to both conditions is determined by a complex interplay of genetic and environmental factors, which centre on an aberrant immune response to microbiota in the gastrointestinal tract.² Emerging evidence from a plethora of different functional and genetic studies clearly indicates both unique and shared pathogenetic mechanisms of critical importance in the genesis and propagation of intestinal inflammation in the two conditions. CD is strongly linked to perturbed recognition and handling of intestinal microbiota by the innate immune system,^{3–7} and a state of impaired bacterial clearance.⁷ Furthermore, a critical role for autophagy, a fundamental catabolic pathway enabling the targeting of cytoplasmic cargo [including organelles and invasive bacteria] to lysosomal compartments for degradation,⁸ is now well established in CD.^{9,10} Autophagy is closely connected with the endoplasmic reticulum [ER] stress response [unfolded protein response, UPR] in highly secretory cells such as Paneth cells localized to the small intestine, with impaired autophagy and non-resolved ER stress coercing to trigger the development of severe spontaneous transmural ileitis.¹¹ Specific deletion of *Atg16l1* in intestinal epithelial cells [IECs] in mice, one of the key autophagy genes associated with CD susceptibility,¹² and homozygosity for the *ATG16L1* risk variant T300A in humans, results in an exaggerated UPR favouring inflammation and cell death, characterized by increased expression of the ER stress sensor IRE1 α in intestinal epithelial crypts as a critical

promoter of ileal inflammation.¹³ Enhanced ER stress is itself a unifying feature of CD and UC epithelium and can be both environmentally and genetically determined, for example by deletion of the UPR transcription factor XBP1.¹⁴

Indeed, there appears to be considerable mechanistic overlap in the pathogenesis of CD and UC, consistent with the high number of concordant genetic susceptibility loci between the two phenotypes,¹⁵ and the efficacy of therapies such as tumour necrosis factor [TNF] blockade in both conditions.^{16,17} A crucial area of shared importance in the pathogenesis of both UC and CD relates to the role of the pro-inflammatory interleukin-23 [IL-23] cytokine. Large genome-wide association studies [GWAS] have identified variants in the *IL23R* gene,¹⁸ encoding the specific receptor for IL-23, and which modify susceptibility to both CD and UC, in common with a number of other autoimmune diseases such as psoriasis and ankylosing spondylitis,^{19,20} pointing to a pivotal role for IL-23 in the pathogenesis of these conditions. First discovered over 20 years ago,²¹ a wealth of evidence now implicates wide-ranging and diverse effects of IL-23 with broad-reaching implications for our understanding of disease pathogenesis and therapeutics. Indeed, clinical therapies antagonizing IL-23 and the closely related cytokine IL-12 are now approved and in mainstream use for the treatment of IBD.²² In this review, we discuss current understanding of fundamental IL-23 biology, explore its pleiotropic effects and the downstream effector pathways contributing to the development of intestinal inflammation, and discuss current and new potential avenues for therapies targeting IL-23 in the treatment of IBD.

2. IL-23: A Pleiotropic Cytokine with Distinct Pro-Inflammatory Effects

IL-23 is a heterodimeric, pro-inflammatory cytokine at the interface of innate and adaptive immunity. It was first reported by Oppmann *et al.* in 2000, who discovered a p19 protein that formed a covalently linked complex with the IL-12p40 subunit, giving rise to the biologically active IL-23 protein.²¹ This interaction distinguishes the molecule from the related cytokine IL-12, in which the shared p40 subunit is covalently linked to a distinct p35 subunit via disulphide bonds to form the prototypical heterodimeric cytokine classically associated with promotion of CD4⁺ Th1 and CD8⁺ cytotoxic responses.²² The IL-12 cytokine family also encompasses the Th1-promoting IL-27 and anti-inflammatory IL-35 cytokines, formed by the EB13/p28 and EB13/p35 heterodimers respectively.^{22,23}

IL-23 exerts its biological effects through engagement with a specific receptor complex highly expressed on the surface of activated CD4⁺ T cells,²¹ group 3 innate lymphoid cells [ILCs],²⁴ and $\gamma\delta$ T cells,²⁵ and also at lower levels on intestinal epithelial cells,²⁶ monocytes and macrophages,²⁷ neutrophils,²⁸ natural killer [NK] cells and invariant NKT cells.²⁹ The IL-23R chain forms a heterodimeric complex with IL-12R β 1 to form the IL-23 receptor.³⁰ The p19 subunit of the IL-23 ligand establishes a critical interaction with the N-terminal immunoglobulin domain of the IL-23R,^{31,32} triggering conformational changes in IL-23 with the p40 subunit concurrently binding to IL-12R β 1, thus bridging the IL-23R and IL-12R β 1 subunits.³² Upon ligand engagement with IL-23R, phosphorylation of Janus kinase 2 [JAK2, a protein constitutively associated with the IL-23R] ensues in parallel with tyrosine kinase 2 [TYK2] and the IL-23R itself, preferentially resulting in phosphorylation, homodimerization and activation of the STAT3 [signal transducer and activator of transcription 3] transcription factor [Figure 1], and to a lesser degree the STAT1, STAT4 and STAT5 transcription factors.³⁰ By contrast, IL-12R signalling, which also requires activation of JAK2 and TYK2, predominantly activates STAT4,³³ mechanistically accounting for the distinct biological effects of the two cytokines.

Functionally, IL-23 plays a role in the host response to pathogens. Mice lacking expression of either IL-23p19 or the shared p40 subunit are unable to clear *Citrobacter rodentium* after oral challenge and succumb to lethal infection.³⁴ Moreover, mice deficient in p40 demonstrate greater susceptibility to *Mycobacterium tuberculosis* infection and compromised antigen-specific interferon γ [IFN γ] responses, compared to either mice lacking p35 subunit expression or wild-type mice.³⁵ Furthermore, absence of p19 increased mortality and diminished IL-17 responses in a *Klebsiella pneumoniae* infection model.³⁶ Concordantly, single nucleotide polymorphisms [SNPs] in the locus containing the *IL23R* gene have been associated with susceptibility to infectious disease such as leprosy,³⁷ and loss of function mutations in the *IL12B* gene [encoding the shared p40 subunit] linked with mycobacterial disease and disseminated Gram-negative bacterial infections.³⁸

3. Genetic, Functional Studies and Interventional Trials Link IL-23 to Intestinal Inflammation

It is well established that CD and UC have a substantial genetic component, and GWAS have identified genetic variants in the *IL23R* region associated with CD and UC susceptibility.^{18,20,39}

A relatively uncommon coding variant [rs11209026] resulting in substitution of arginine for glutamine at position 381 [R381Q] is markedly protective against the development of IBD in Caucasian populations.¹⁸ The R381Q variant is localized to the cytoplasmic region of the IL-23R protein, close to the binding site of JAK2, and leads to hypomorphic function.⁴⁰ The presence of glutamine at position 381 decreases stability of the IL-23R protein, thereby diminishing receptor expression at the cell surface and thus STAT3/STAT4 activation in response to IL-23.⁴¹ Targeted resequencing of susceptibility loci identified two further protective rare coding variants in the *IL23R* gene, resulting in amino acid substitutions G149R and V362I, both of which have an allele frequency of <2% in healthy populations and similarly result in loss of function.^{42,43} Moreover, non-coding variants in intronic regions of the *IL23R* gene have been associated with IBD susceptibility independently of rs11209026 [R381Q].¹⁸

Reinforcing the importance of IL-23 in the pathogenesis of IBD, common variants in regions neighbouring the *IL12B* gene, which encodes the shared p40 subunit of IL-12 and IL-23, are associated with IBD susceptibility.⁴⁴ Meta-analysis concordantly identified associated SNPs in regions containing the *JAK2*, *TYK2* and *STAT3* genes as shared risk factors for CD and UC.¹⁵ As discussed above, the products of these genes are important in intracellular signalling transduction downstream of IL-23. Identifying causal variants and understanding their precise impact on immune cell biology remains a considerable challenge, given that many disease-associated SNPs localize to non-coding genomic regions and exist in linkage disequilibrium with other polymorphisms. Fine mapping of the *TYK2* genomic locus with SNP imputation and haplotype inference, using data across five different autoimmune diseases including CD and UC, has identified the probable causal variant in this region as rs34536443⁴⁵; the protective coding variant results in an amino acid substitution [P1104A] in the *TYK2* catalytic kinase domain. Whilst this protective variant indeed diminishes STAT3 phosphorylation in memory T cells upon IL-23 receptor stimulation,⁴⁵ pleiotropic effects including on type I IFN receptor signalling, in which *TYK2* plays a shared role,^{45,46} should also be noted. This may explain the broad-ranging associations of rs34536443 with other seemingly distinct autoimmune diseases.^{47,48}

Alongside the genetic evidence, experimental data from a plethora of animal models corroborate a critical role for IL-23 in the generation of intestinal inflammation. Increased levels of IL-23p19 in inflamed mucosa are a consistent feature of experimental colitis.⁴⁹ Mice deficient in p19, but not the p35 subunit of IL-12 [which is notably also shared with IL-35], are protected from the spontaneous enterocolitis that occurs upon genetic deletion of the anti-inflammatory cytokine IL-10.⁵⁰ In adoptive transfer colitis models, in which naive CD4⁺ CD45RB^{high} T cells are transferred into syngeneic immunodeficient mice,⁵¹ IL-23 unequivocally promotes inflammation. The colitis induced following adoptive T cell transfer was markedly diminished in recipient mice lacking expression of the shared p40 or IL-23p19 subunits, but not the p35 subunit.⁴⁹ Genetic absence of the IL-23R in the adoptively transferred CD4⁺ CD45RB^{high} T cells diminished their colitogenic potential, corroborating the importance of IL-23.⁵² Furthermore, anti-IL-23p19 treatment also prevented the development of colitis in SCID mice upon transfer of caecal bacterial antigen-specific CD4⁺ T cells, and furthermore ameliorated established colitis in this model.⁵³ Contrastingly,

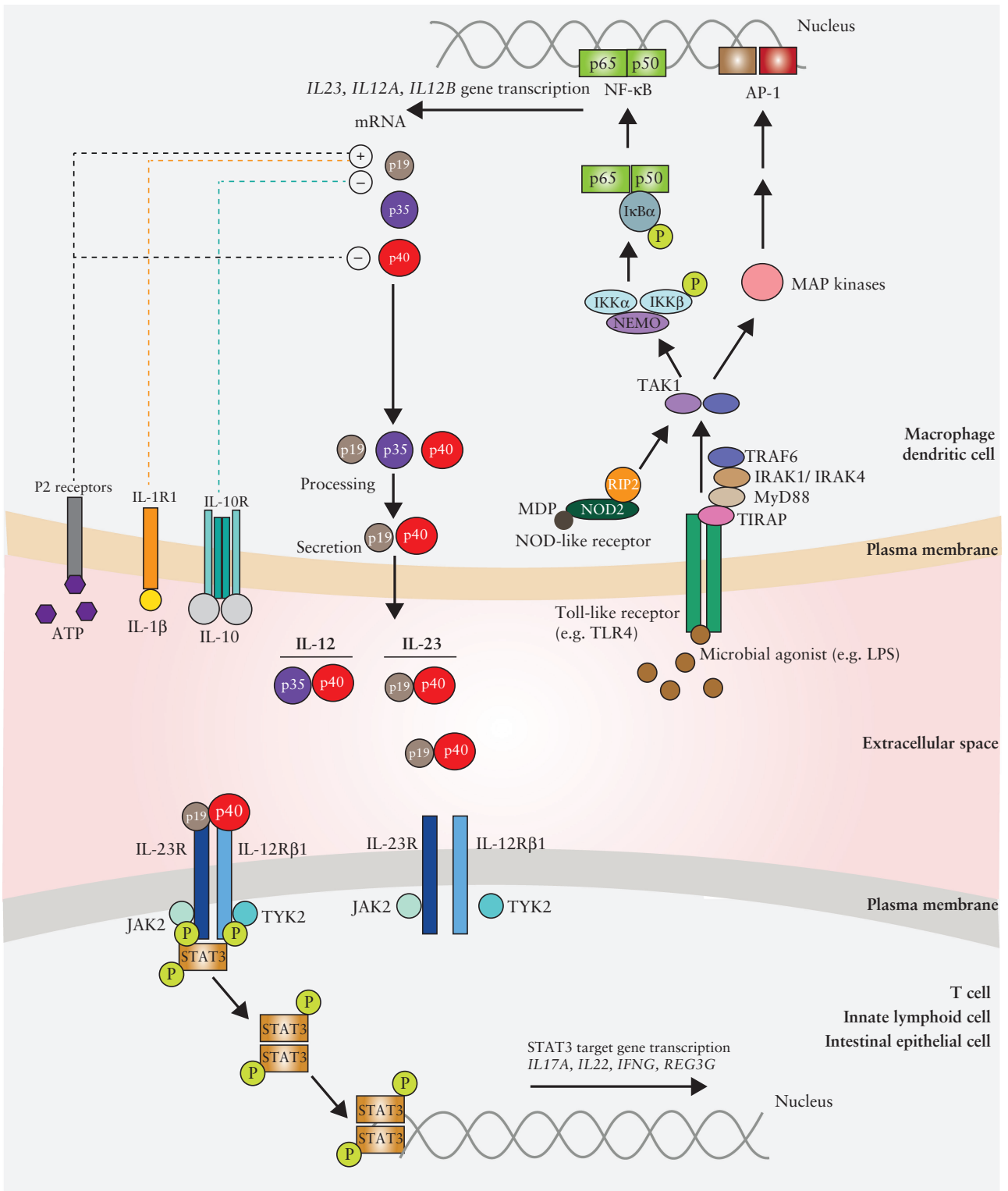


Figure 1. Generation of the pro-inflammatory cytokine IL-23 and its downstream signalling via the IL-23 receptor. Schematic depicts the production of IL-23 by macrophages and dendritic cells and its effects via the IL-23 receptor. In response to stimulation of Toll-like receptors and NOD-like receptors by microbial agonists, activation of intracellular signalling cascades ensues, resulting in activation of transcription factors such as NF-κB and AP-1. This enables cytokine gene transcription, including *IL23A*, *IL12A* and *IL12B*, which encode the p19, p35 and p40 subunits of the IL-12 and IL-23 cytokines respectively. Further critical regulatory signals modulating IL-23 release include cytokines such as IL-1β and IL-10 and adenosine nucleotides such as ATP, signalling via specific cell surface receptors. IL-23, a heterodimeric protein comprising the p19 and shared p40 subunit with IL-12, binds its cognate receptor expressed on the surface of cells such as T lymphocytes, innate lymphoid cells and intestinal epithelial cells. This engagement results in phosphorylation of JAK2 and TYK2 associated with the IL-23R and subsequently phosphorylation, homodimerization and activation of STAT3. This consequently triggers induction of STAT3 target genes such as *IL17A*, *IL22*, *IFNG* and *REG3G*.

in TNBS-induced colitis, deficiency of IL-23p19 surprisingly enhanced the severity of colitis.⁵⁴ This may relate to enhanced IL-12 production by dendritic cells [DCs] due to loss of counter-regulation when IL-23p19 is absent, as neutralization of p40 improved features of intestinal inflammation in this context.⁵⁴

IL-23 is essential in murine colitis models dependent on the innate immune system. Colitis triggered by the pathogenic bacterium *Helicobacter hepaticus* in immunodeficient mice was attenuated by treatment with an anti-IL-23p19 antibody.⁴⁹ TRUC mice [*T-bet*^{-/-} × *Rag2*^{-/-}], which lack expression of the T-box transcription factor T-bet as well as mature T and B lymphocytes, develop a spontaneous distal continuous colitis strikingly akin to human UC,⁵⁵ which is dependent on innate lymphoid cells [discussed below] and the intestinal microbiota.^{55,56} Anti-IL-23p19 administration dramatically improved spontaneous colitis in the TRUC model compared to isotype controls.⁵⁶ Treatment of T and B cell-deficient *Rag1*^{-/-} mice with a stimulatory anti-CD40 antibody triggers a distinctive innate immune-dependent colitis and a systemic wasting disease associated with elevated serum pro-inflammatory cytokine levels.⁵⁷ The mucosal inflammation observed is dependent on IL-23 production, which is upregulated in colonic DCs and monocytes upon CD40 stimulation, as co-treatment with a neutralizing anti-IL-23p19 antibody dramatically abrogated the development of colitis.⁵⁷ Intriguingly, features of systemic disease observed in this model were dependent on IL-12 rather than IL-23, highlighting important mechanistic differences in the generation of localized mucosal vs systemic inflammatory responses.⁵⁷

The effective translation of therapies directed against IL-23 into clinical practice moreover substantiates the detrimental role played by this cytokine in IBD pathogenesis. Biological therapies targeting the shared p40 subunit of IL-12 and IL-23 were initially evaluated in CD, stemming from the classical paradigm of CD as a Th1-associated autoimmune disease driven by IL-12 production by DCs.²² The first phase 2 study of a targeted monoclonal anti-p40 treatment [ABT-874] in active CD indicated a significantly improved rate of clinical response compared to placebo following 7 weeks of treatment, which was associated with a reduction in levels of IL-12, TNF α and IFN γ released by stimulated lamina propria mononuclear cells isolated from colonic biopsies.⁵⁸ Subsequently, clear-cut evidence of the efficacy of p40 blockade in CD, in the form of the fully humanized IgG1 monoclonal ustekinumab, was established in the phase 3 UNIFI studies.⁵⁹ A significantly enhanced rate of clinical response, based on Crohn's disease activity index [CDAI] score, was observed in ustekinumab-treated groups compared to placebo, regardless of preceding anti-TNF [UNIFI-1 cohort] or conventional immunomodulatory therapy [UNIFI-2 cohort] failure. The higher clinical response rate corresponded to a more pronounced reduction in faecal calprotectin and serum C-reactive protein levels,⁵⁹ as well as improved endoscopic activity at 8 weeks in a sub-study of participants receiving ustekinumab compared to placebo.⁶⁰ Furthermore, in the maintenance IM-UNIFI study, in which ustekinumab responders were continued on ustekinumab or placebo treatment, 53.1 and 48.8% of patients achieved clinical remission in the 8 and 12 weekly treatment groups respectively vs 35.9% of the placebo arm after 44 weeks of treatment.⁵⁹ Concordantly, inhibition of the p40 subunit is also an effective therapeutic strategy in UC. The phase 3 UNIFI study recently demonstrated the efficacy of ustekinumab in the induction and maintenance of

remission in a cohort of patients with moderate-to-severely active disease.⁶¹

Given that ustekinumab targets the shared p40 unit, the unequivocal clinical efficacy observed in the UNIFI and UNIFI studies could potentially be explained by either IL-12 or IL-23 inhibition, or a combination of both. Considering the colitogenic role of IL-23 demonstrated in preclinical studies, and the unambiguous genetic evidence tying the *IL23R* gene with IBD susceptibility, it would be logical to hypothesize that selective p19 blockade would be equally efficacious and potentially advantageous. Brazikumab [formerly named MEDI2070] is a humanized IgG2 monoclonal antibody that binds p19,⁶² thus offering selective inhibition of IL-23. A phase 2a study of brazikumab in a cohort of patients with active CD refractory to TNF inhibition indeed showed improved rates of clinical response at 8 weeks compared to placebo, although endoscopic outcomes were not assessed.⁶³ Risankizumab is another humanized monoclonal IgG1 developed against the p19 subunit. A multicentre, randomized controlled phase 2 study of patients with active CD indeed revealed superiority of risankizumab over placebo in the induction of clinical remission after 12 weeks of therapy.⁶⁴ Extended induction treatment further enhanced the clinical response and remission rate after 26 weeks, which was sustained at 52 weeks for the majority of patients in the open label maintenance phase of the study.⁶⁵

Mirikizumab [LY3074828], a distinct humanized monoclonal IgG4 antibody that neutralizes the p19 subunit, was recently evaluated in phase 2 studies in UC and CD.^{66,67} In the UC study, patients were assigned to receive 50, 200 or 600 mg mirikizumab or placebo; in former two treatment arms dose escalation based on plasma drug levels was conducted. Although the difference in primary outcome, clinical remission between the high-dose mirikizumab and placebo group at 12 weeks, was not statistically significant, improved clinical response rates were seen in all treatment groups which reached nominal statistical significance compared to the placebo arm.⁶⁶ In CD, treatment with either 200, 600 or 1000 mg mirikizumab was associated with a higher rate of endoscopic response following 12 weeks of treatment compared to placebo.⁶⁷ Overall, selective targeting of p19 thus appears to be a promising therapeutic strategy in both CD and UC [details of recent and current phase 2 and 3 studies are summarized in Tables 1 and 2].

It could also be envisaged that targeting IL-23R directly, or its downstream signalling molecules such as JAK2 and TYK2, might be correspondingly efficacious. Tofacitinib, an orally active pan-JAK inhibitor with weaker inhibitory effects on TYK2, has demonstrated efficacy in the induction and maintenance of remission in UC in phase 3 studies compared to placebo⁶⁸ and is now approved for use in UC. Originally developed as a JAK3 inhibitor, tofacitinib has been noted to exert suppressive effects on Th17 cell differentiation *in vitro*, and to diminish IL-23R expression, alongside other broad-ranging immunomodulatory effects.⁶⁹ Further more selective small molecules targeting TYK2 are presently under evaluation in both CD and UC, several of which have concurrent inhibitory effects on Janus kinases.⁷⁰ Brepocitinib [PF-06700841], an orally administered dual inhibitor of JAK1 and TYK2, potently inhibits STAT3 activation in response to IFN α , IL-23 and IL-27 stimulation of whole blood, and furthermore diminishes STAT4 activation in response to IL-12.⁷¹ This compound is currently under investigation in phase 2 studies of moderate to severe CD [NCT03395184]

Table 1. Summary of novel therapies targeting IL-23 and the IL-23R signalling pathway in Crohn's disease. Summary of novel therapies targeting IL-23 and its downstream signalling pathway with details of key phase 2 and phase 3 studies in Crohn's disease [shaded area indicates phase 2 and 3 studies currently in progress or not yet reported in full]

Target	Drug	Evidence	Number of included patients	Sex and mean age of included patients	Key primary and secondary outcomes [or anticipated outcomes]	Reference or ClinicalTrials.gov identifier
Shared IL-12/IL-23 p40 subunit	Briakinumab [ABT-874]	Phase 2 study	<p><i>n</i> = 79</p> <p>Cohort 1 <i>n</i> = 40</p> <p>Cohort 2 <i>n</i> = 39</p>	<p>Cohort 1: M/F <i>n</i> = 12/28</p> <p>Mean age:</p> <p>36.9 years [placebo]</p> <p>43.1 years [1 mg/kg group]</p> <p>42.5 years [3 mg/kg group]</p> <p>Cohort 2 M/F <i>n</i> = 14/25</p> <p>Mean age:</p> <p>41.0 years [placebo]</p> <p>39.9 years [1 mg/kg group]</p> <p>39.8 years [3 mg/kg group]</p>	<p>Primary: safety evaluation of use in Crohn's disease.</p> <p>Secondary: superior clinical response [fall in CDAI score of >100 at week 7] vs placebo.</p>	Mannon et al. <i>N Engl J Med</i> 2004;351[20]:2069-79
				<p>UNITH-1: M/F <i>n</i> = 317/424</p> <p>Mean age:</p> <p>37.3 years [placebo]</p> <p>37.4 years [130 mg group]</p> <p>37.3 years [6 mg/kg group]</p> <p>UNITH-2: M/F <i>n</i> = 293/335</p> <p>Mean age:</p> <p>40.2 years [placebo]</p> <p>39.1 years [130 mg group]</p> <p>38.4 years [6 mg/kg group]</p> <p>IM-UNITH: M/F 173/224</p> <p>39.5 years [placebo]</p> <p>37.9 years [12 weekly group]</p> <p>38.6 [8 weekly group]</p>	<p>UNITH-1:</p> <p>Significantly enhanced clinical response [fall in CDAI score of >100, or CDAI <150] at week 6 vs placebo.</p> <p>Response rate: 34.3% [130 mg group], 33.7% [6 mg/kg group] vs 21.5% [placebo].</p> <p>UNITH-2:</p> <p>Significantly improved clinical response rate at week 6 vs placebo.</p> <p>Response rate: 51.7% [130 mg group], 55.5% [6 mg/kg group] vs 28.7% [placebo].</p> <p>IM-UNITH:</p> <p>Significantly improved rate of clinical remission [CDAI < 150] at week 44 of treatment vs placebo.</p> <p>Remission rate: 53.1% [8 weekly group], 48.8% [12 weekly group] vs 35.9% [placebo group].</p> <p>Significantly improved clinical response rate [reduction in CDAI score of at least 100, or CDAI <150] after 8 weeks of treatment [49.2%] compared to placebo [26.7%].</p>	Feagan et al. <i>N Engl J Med</i> 2016;375[20]:1946-60
IL-23 p19 subunit	Brazikumab [MED12070]	Phase 2a randomized placebo-controlled trial	<i>n</i> = 119	<p>M/F <i>n</i> = 45/74</p> <p>Mean age:</p> <p>38.1 years [placebo]</p> <p>34.9 years [MED12070]</p>	<p>Significantly higher rate of clinical remission [CDAI < 150] in 200 mg and 600 mg pooled treatment groups [30.5%] after 12 weeks of treatment vs placebo [15.4%].</p>	Sands et al. <i>Gastroenterology</i> 2017;153[1]:77-86.e6.
	Risankizumab	Phase 2 randomized placebo-controlled trial	<i>n</i> = 121	<p>Mean age:</p> <p>36 years [placebo]</p> <p>39 years [pooled risankizumab groups]</p>	<p>Significantly higher rate of clinical remission [CDAI < 150] in 200 mg and 600 mg pooled treatment groups [30.5%] after 12 weeks of treatment vs placebo [15.4%].</p>	Feagan et al. <i>Lancet</i> 2017;389:1699-709.
	Mirikizumab	Phase 2 randomized placebo-controlled trial [SERENITY]	<i>n</i> = 191	<p>Mean age:</p> <p>38.1 years [200 mg group]</p> <p>40.4 years [600 mg group]</p> <p>37.7 years [1000 mg group]</p>	<p>Higher percentage of participants achieving at least a 50% decrease in simple endoscopic activity score-Crohn's disease [SES-CD] in all treatment groups compared to placebo [reaching pre-specified significance threshold of 0.1].</p>	Sands et al. <i>Gastroenterology</i> 2022;162:495-508.
IL-23 receptor	JNJ-67864238	Phase 2 randomized placebo-controlled trial [PRISM]	<i>n</i> = 48		<p>Difference in CDAI after 12 weeks of treatment compared to baseline.</p> <p>Study terminated early as futility criteria met.</p>	NCT04102111

Table 1. Continued

Target	Drug	Evidence	Number of included patients	Sex and mean age of included patients	Key primary and secondary outcomes [or anticipated outcomes]	Reference or Clinicaltrials.gov identifier
IL-23 p19 subunit	Risankizumab	Phase 3 randomized placebo-controlled trials [MOTIVATE and ADVANCE]	n = 931 [NCT03105128] n = 618 [NCT03104413]		Percentage with moderate–severely active disease achieving clinical remission at week 12 [CDAI]; and proportion achieving endoscopic response.	NCT03105128 NCT03104413
	Guselkumab	Phase 2/3 randomized placebo and active controlled trial [GALAXI]	Estimated n ~2000		Difference in CDAI after 12 weeks of treatment compared to baseline [phase 2 study]. Clinical remission [CDAI < 150] and endoscopic response after 12 weeks of treatment [phase 3 study].	NCT03466411
JAK1/TYK2	Brepocitinib	Phase 2 randomized placebo-controlled trial [NCT03395184]	Estimated n ~250		Proportion of patients achieving improvement in endoscopic activity [50% reduction in SES-CD].	NCT03395184
TYK2	Deucravacitinib	Phase 2a randomized placebo-controlled trial [LATTICE-CD]	Estimated n ~240		Percentage of patients in clinical remission and percentage demonstrating endoscopic response after 12 weeks of treatment.	NCT03599622

and UC [NCT02958865], having been generally well tolerated and showing clinical benefit in plaque psoriasis.⁷² Deucravacitinib [BMS-986165] is a novel allosteric inhibitor selective for TYK2, which binds the pseudokinase protein domain and thereby stabilizes autoinhibitory interactions with the neighbouring functional catalytic site.^{73,74} Biologically, deucravacitinib dampens STAT3 phosphorylation in CD161⁺ CD3⁺ Th17 cells in response to IL-23 stimulation, together with functional effects on B cells and monocyte differentiation attributed to concurrent blockade of type I IFN signalling.⁷⁵ *In vivo*, deucravacitinib protected SCID mice from excessive weight loss in both anti-CD40-induced colitis and following adoptive transfer of CD4⁺CD45RB^{high} T cells; histological analysis of colonic tissue demonstrated a favourable effect of deucravacitinib equivalent to that of anti-p40 treatment in both colitis models.⁷⁵ Deucravacitinib has now entered phase 2 studies in CD [LATTICE-CD, NCT03599622] and UC [NCT04613518] [see also Tables 1 and 2]. Pharmacologically targeting the IL-23R itself has not yet successfully materialized in IBD; a phase 2 study of JNJ-67864238, a locally acting oral peptide antagonizing IL-23R, was recently terminated after meeting criteria for futility [NCT04102111]. It will be interesting to determine whether the efficacy of inhibitors targeting IL-23R signalling is influenced by the presence of protective or disease-associated variants in *IL23R* and related genes.

4. Macrophages and DCs Release IL-23 Upon Microbial Stimulation

Macrophages and antigen presenting cells are the principal cellular sources of IL-23.²¹ In healthy non-inflamed mucosa, expression of IL-23 is greatest in the terminal ileum; lamina propria CD11c⁺ DCs in this region demonstrate constitutive expression of the shared p40 subunit in the presence of intestinal microbiota.⁷⁶ IL-23p19 expression is increased in inflamed mucosa from patients with both UC and CD, derived predominantly from CD68⁺ macrophages and DCs.⁷⁷ In the inflamed colon, tissue-infiltrating neutrophils also appear to contribute to IL-23 production.⁷⁸ In CD, IL-23p19 mRNA levels are positively correlated with the severity of macroscopic lesions identified at endoscopy.⁷⁹ Lamina propria CD14⁺ macrophages expressing IL-23p19 were also noted to be more frequent in CD patients with disease refractory to TNF antagonism, which was associated with accumulation of TNFR2⁺ IL-23R⁺ CD4⁺ T cells resistant to apoptosis in the intestine.⁸⁰

DCs and macrophages upregulate IL-23 production upon encountering microbial stimuli. In the intestine, lamina propria CD14⁺ CD33⁺ macrophages have a particularly high propensity for IL-23 secretion in response to commensal bacteria such as *Escherichia coli* and *Enterococcus faecalis*, the abundance of which is increased in CD patients compared to controls.^{81,82} Stimulation of macrophages and DCs with antigens such as Toll-like receptor [TLR] agonists initiates intracellular signalling cascades culminating in increased *IL23A* gene transcription and protein secretion. Interestingly, myeloid DCs isolated from mesenteric lymph nodes of patients with CD were noted to secrete quantitatively higher levels of the shared p40 subunit and IL-23p19 in response to *Enterococcus faecalis* extract compared to patients with UC.⁸³

The potency of IL-23 induction by macrophages and DCs in response to antigenic stimulation is determined by a

Table 2. Summary of novel therapies targeting IL-23 and the IL-23R signalling pathway in ulcerative colitis. Summary of novel therapies targeting IL-23 and its downstream signalling pathway with details of key phase 2 and phase 3 studies in ulcerative colitis [shaded area indicates phase 2 and 3 studies currently in progress or not yet reported in full]

Target	Drug	Evidence	Number of included patients	Sex and age range of included patients	Summary of primary outcomes, or anticipated primary outcomes if not yet reported	Reference or Clinicaltrials.gov identifier
Shared IL-12/IL-23 p40 subunit	Ustekinumab	Phase 3 randomized placebo-controlled trials [UNIFI study].	<i>n</i> = 961 [induction study]	Induction trial: M/F <i>n</i> = 582/379 Mean age: 41.2 years [placebo group] 42.2 years [130 mg group] 41.7 years [6 mg/kg group]	Induction trial: clinical remission significantly higher after 8 weeks of treatment [15.6%, 130 mg group and 15.5%, 6 mg/kg group] compared to placebo [5.3%]. Maintenance trial: significantly enhanced rate of clinical remission after 44 weeks [in 8 and 12-weekly treatment arms] compared to placebo.	Sands et al. <i>N Engl J Med</i> 2019;381:1201–14
			<i>n</i> = 523 [maintenance study]	Maintenance trial: M/F <i>n</i> = 297/226 Mean age: 42.0 years [placebo] 40.7 years [12 weekly group] 39.5 years [8 weekly group]		
IL-23 p19 subunit	Mirikizumab	Phase 2 randomized placebo-controlled trial	<i>n</i> = 249	M/F <i>n</i> = 149/100 Mean age: 42.6 years [placebo] 41.8 years [50mg] 43.4 years [200 mg] 42.4 years [600 mg]	Clinical remission after 12 weeks of treatment compared to placebo—difference not statistically significant.	Sandborn et al. <i>Gastroenterology</i> 2020;158:537–49 e10
IL-23 p19 subunit	Mirikizumab	Phase 3 randomized placebo-controlled trial [LUCENT 1, LUCENT 2]	Estimated ~1160 participants [NCT03518086]		Clinical remission [determined using modified Mayo score] after 12 weeks of treatment [LUCENT 1].	NCT03518086
			Estimated ~1044 participants [NCT03524092]		Clinical remission after 40 weeks of treatment [LUCENT 2].	NCT03524092
	Risankizumab	Phase 3 randomized placebo-controlled study	Estimated ~942 participants		Percentage of participants achieving clinical remission at week 52 [determined using adapted Mayo score].	NCT03398135
	Guselkumab	Phase 2b/3 randomized placebo-controlled trial [QUASAR]	Estimated ~1000 participants		Clinical response [induction study 1] after 12 weeks of treatment Clinical remission [induction study 2].	NCT04033445
Brazikumab	Phase 2 study	Estimated ~256 participants		Clinical remission after 44 weeks of treatment [maintenance study]. Clinical remission [defined by modified Mayo score] after 10 weeks of treatment.	NCT03616821	

Table 2. Continued

Target	Drug	Evidence	Number of included patients	Sex and age range of included patients	Summary of primary outcomes, or anticipated primary outcomes if not yet reported	Reference or Clinicaltrials.gov identifier
JAK1/ TYK2	Brepocitinib	Phase 2b randomized, placebo-controlled study	Estimated ~319 participants		Total Mayo score of participants after 8 weeks of treatment.	NCT02958865
TYK2	Deucravacitinib	Phase 2 randomized, placebo-controlled trial	Estimated ~131 participants		Clinical remission according to modified Mayo score following 12 weeks of treatment.	NCT03934216

complex interplay of factors, some intrinsic to the cell and others related to the surrounding environmental milieu. First, the nature of the microbial stimulus and specific pattern recognition receptors engaged critically determines IL-23 production relative to other cytokines such as IL-12,⁸⁴ bacterial peptidoglycan stimulation for example preferentially inducing IL-23p19 over IL-12p35 *in vitro*,^{85,86} whereas the TLR4 agonist lipopolysaccharide [LPS] demonstrates capacity for both IL-12 and IL-23 upregulation, especially in the presence of IFN γ .^{87,88} Stimulation of the cytosolic nucleotide-binding oligomerization domain-containing protein 2 [NOD2] receptor, the protein encoded by the earliest identified CD susceptibility gene,^{3,4} augments IL-23 production by DCs and IL-17 production in DC and CD4⁺ T cell co-cultures. DCs expressing hypomorphic NOD2 variants were initially shown to have diminished capacity for IL-23 production, with loss of NOD2 in DCs leading to reduced IL-17 induction by CD4⁺ memory T cells.⁸⁶ Somewhat paradoxically, subsequent work using activated DCs showed that NOD2 variants linked to CD also associate with decreased expression of miR-29, a microRNA capable of indirectly downregulating IL-23p19 via effects on ATF2.⁸⁹ miR-29 expression in DCs itself reportedly diminished IL-23p19 production and Th17 responses in co-culture experiments. Thus, the presence of NOD2 disease-associated variants is predicted to enhance IL-23 responses, and indeed these variants were noted to augment p40 secretion from DCs upon adherent invasive *Escherichia coli* stimulation.⁸⁹ Nonetheless, these studies clearly highlight the importance of microbe and innate immune interactions, influenced by host genetic factors, in the initial generation and propagation of the IL-23 response.

The IL-23 response is further shaped by a complex network of pro- and anti-inflammatory cytokines and other biologically active molecules. In particular, recent work identified release of IL-10 by a specific population of peripheral blood mononuclear cells [PBMCs] as an important paracrine regulator of IL-23p19 production by distinct CD14⁺ mononuclear cells.⁹⁰ The inhibitory effect of IL-10 on pro-inflammatory cytokine production was abrogated in patient cells harbouring *IL10R* mutations linked to IBD, whereas IL-1R1 blockade was effective in dampening IL-23p19 expression.⁹⁰ Thus, IL-10 and IL-1 β are key negative and positive regulators of IL-23 responses, respectively. This raises the possibility that IL-1 receptor antagonism, currently under investigation in acute severe UC,⁹¹ might be effective in part by inhibiting the IL-23 response in individuals with *IL10R* mutations, or in those with a state of induced 'functional resistance' in IL-10R signalling occurring upon bacterial exposure proposed by the authors.⁹⁰

Beyond cytokines, small molecules such as extracellular adenosine nucleotides, known to accumulate at sites of inflammation and be released under conditions of cell stress,⁹² enhance IL-23p19 induction by DCs in response to *Escherichia coli* via P2 purinergic receptor signalling whilst reciprocally suppressing IL-12 responses.⁹³ Intracellular metabolism within DCs and macrophages appears to provide a further level of regulation in the IL-23 response. Incubation of murine bone-marrow-derived DCs with palmitic acid, a saturated fatty acid, enhanced expression of IL-23p19 following TLR stimulation.⁹⁴ Mechanistically, palmitic acid was reported to inhibit the glycolytic enzyme hexokinase, leading to diminished glycolytic flux, elevated mitochondrial reactive oxygen species production and activation of the UPR to

trigger IL-23 transcription.⁹⁴ Although experimental colitis was not investigated in that study, mice fed a high-fat diet did demonstrate exacerbated psoriasis-like inflammation in response to topical imiquimod application, with an enhanced number of IL-23⁺ cDC1s present in regional lymph nodes in a manner dependent on DC expression of XBP1.⁹⁴ Exogenous dietary components may therefore directly shape metabolic rewiring of immune cells to control IL-23 generation and autoimmunity. Of further note, several of the IBD-associated genes encode proteins crucially involved in metabolism.^{95,96} This includes FAMIN, an evolutionary conserved multifunctional enzyme that through its core catalytic activities enables a purine nucleotide cycle in macrophages.⁹⁷ It would therefore be interesting to explore whether additional levels of immunometabolic regulation on the IL-23 response might exist, and how these are influenced by host genetic factors. In all, modulation of the IL-23 response by myeloid cells might therefore be accomplished through therapeutic targeting of regulatory cytokines and small molecules produced by the host, components of diet, the intestinal microbiome and its associated rich array of metabolites, and the downstream intracellular signalling pathways that they regulate.

5. Effectors of the IL-23 Response

5.1. The IL-23–Th17 axis

A major focus of the pro-inflammatory effects of IL-23 has centred on its impact on Th17 cells. Th17 cells are a subset of CD4⁺ T helper cells first identified in 2005,⁹⁸ characterized by expression of the retinoic acid-related orphan receptor γ [ROR γ t] transcription factor and their signature cytokine IL-17 [also known as IL-17A], alongside others including IL-17F, IL-22 and TNF α . These cells are important in the host response to extracellular bacterial and fungal infections, although conversely play a pathogenic role in various autoimmune diseases.⁹⁹ Th17 cells are present in the intestinal lamina propria under conditions of homeostasis and constitutively produce IL-17A.¹⁰⁰ A significant increase in Th17 cells is observed in inflamed CD and UC mucosa compared to controls.¹⁰¹ Animal studies have revealed the importance of intestinal microbiota composition in Th17 cell induction within the intestine, and interestingly the colonization of mice with specific Gram-positive commensals known as segmental filamentous bacteria appears sufficient for Th17 cell induction.¹⁰²

IL-23 does not appear to induce Th17 differentiation or IL-17A production from naïve CD4⁺ T cells per se, given that these cells lack significant expression of the IL-23R.²¹ The differentiation of Th17 cells from naïve CD4⁺ T cells requires distinct cytokines such as IL-6 and transforming growth factor β [TGF β], although IL-23 does seem to have a role in their continued survival and expansion.¹⁰³ Treatment of naïve CD4⁺ T cells with IL-6 and IL-21 in the presence of low concentrations of TGF β *in vitro* suppresses expression of the transcription factor Forkhead box P3 [FoxP3], reciprocally enhancing ROR γ t,¹⁰⁴ and furthermore synergistically upregulates expression of the IL-23R.^{104,105} This allows enhanced IL-23R signalling, which may further promote IL-17 production [Figures 2 and 3].¹⁰⁵ Naïve CD4⁺ T cells from healthy volunteers heterozygous for the IBD-protective R381Q variant, isolated and differentiated towards a Th17-like phenotype, released significantly less IL-17A in response to IL-23 stimulation compared to those homozygous for the common risk variant.¹⁰⁶ Concordantly,

memory CD4⁺ CD45RO⁺ cells [in which IL-23R expression is upregulated²¹] from healthy individuals harbouring the R381Q variant also produced significantly lower amounts of IL-17 and IL-22 upon activation and stimulation with IL-23.¹⁰⁷ Furthermore, isolated memory CD4⁺ T cells from individuals homozygous for the protective variant also demonstrated reduced IL-17A and IL-17F production in response to IL-23 in another study, compared to those homozygous for the risk allele.¹⁰⁸

Based on the above observations, it would be tempting to extrapolate that IL-17, derived from Th17 cells, might be a major pathogenic factor in IBD amenable to therapeutic targeting. Despite clear experimental evidence that IL-17 plays a critical function in the host pathogen response, as well as in the development of autoimmune diseases such as arthritis and psoriasis,¹⁰⁹ its role in the development of IBD is more controversial. First, data from animal models of colitis are rather conflicting. Neither inhibition of the IL-17 receptor nor neutralization of IL-17A and IL-17F had any appreciable effect in innate anti-CD40-induced colitis.²⁴ In T cell transfer models of colitis, genetic absence of IL-17A in naïve CD4⁺ CD45RB^{high} T cells did not impair their ability to induce colitis in immunodeficient hosts,^{110,111} and was even associated with more severe inflammatory disease in one study.¹¹² Genetic absence of IL-17A was also reported to increase the severity of pathology in chemically induced colitis,¹¹³ but made no difference or improved disease activity parameters and survival in other studies.^{114–116} Administration of a neutralizing anti-IL-17 antibody significantly worsened acute dextran sodium sulphate [DSS]-induced colitis in mice.¹¹⁷ The exacerbated disease activity may in part reflect the loss of protective effects of IL-17A in the maintenance of gut epithelial integrity and reducing intestinal permeability.^{113,114}

Following from these studies, clinical targeting of IL-17 has not proven effective in the treatment of CD, despite showing striking benefit in other diseases such as psoriasis.¹¹⁸ Secukinumab, a neutralizing human anti-IL-17A, was ineffective in a proof-of-concept study of moderate to severe CD.¹¹⁹ A further human anti-IL-17 receptor A monoclonal antibody, brodalumab, caused significant deterioration of CD in patients with active disease.¹²⁰

Could alternative mechanisms thus explain how Th17 cells contribute to disease? Th17 cells co-express cytokines such as GM-CSF and IL-22 [discussed below], which are induced in the presence of IL-23.^{121,122} Furthermore, Th17 cells themselves demonstrate considerable plasticity and phenotypic heterogeneity.^{123,124} In the appropriate milieu, they can morph into a phenotype with a closer resemblance to Th1 cells, characterized by IFN γ production.^{125,126} Restimulation of Th17-polarized CD4⁺ T cells in the presence of either IL-12 or IL-23 *in vitro* actually appears to diminish IL-17A and IL-17F and enhance IFN γ expression over time, when levels of TGF β are low or absent.¹²⁷ *In vivo*, adoptive transfer of naïve CD4⁺ CD45RB^{high} cells deficient in IL23R expression into *Rag1*^{-/-} mice reduced the proportion of IL-17A⁺ IFN γ ⁺ cells in the colon, compared to recipients that received wild-type naïve cells, as a consequence of diminished proliferation in the intestine.⁵² Thus, IFN γ -producing Th17 cells, potentiated by IL-23, could represent a critical pathogenic cell type in IBD. In support of this, transfer of IL-17⁺ Th17-differentiated precursor cells into *Rag1*^{-/-} mice results in an accelerated and severe colitis, which is contingent on IFN γ expression by the precursor cells.¹²⁸ Indeed enrichment of IFN γ ⁺ IL-17A⁺ CD4⁺

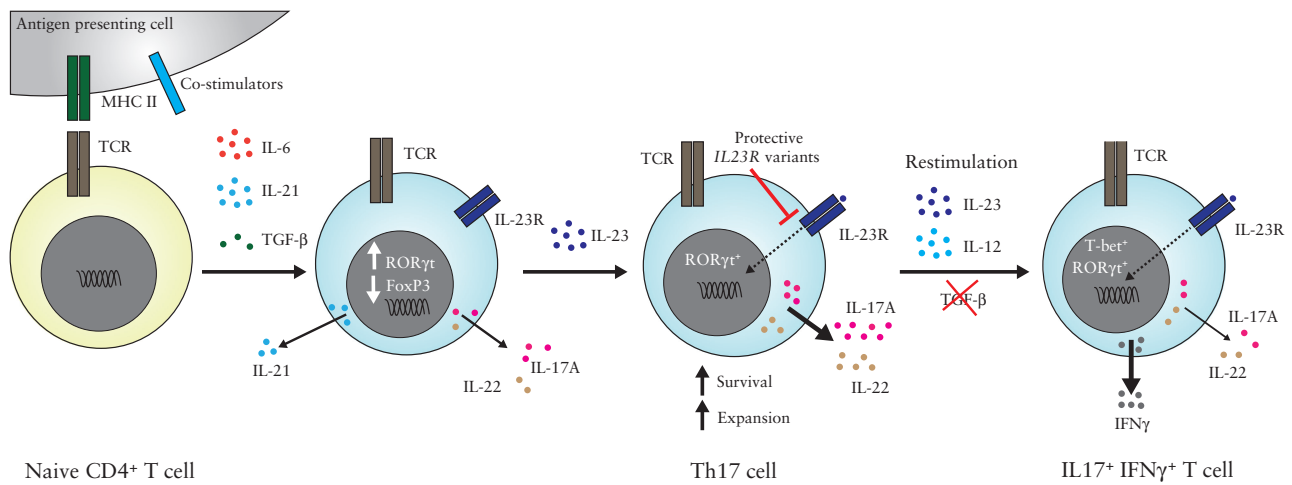


Figure 2. The IL23/Th17 axis. Naive CD4⁺ T cells, which lack expression of the IL-23 receptor, polarize towards a Th17 phenotype upon encountering specific signals from antigen presenting cells and cytokines such as IL-6, IL-21 and TGF- β . This results in the reciprocal up- and down-regulation of the signature ROR γ t and FoxP3 transcription factors respectively, alongside increased IL-23 receptor expression. IL-23 signalling via its cognate receptor promotes the survival and expansion of IL-17 and IL-22-producing Th17 cells, diminished by the presence of variants in *IL23R* conferring protection against autoimmune disease. Upon restimulation when IL-23/IL-12 levels are high, Th17 cells can morph into a Th1-like phenotype characterized by expression of the transcription factor T-bet and production of the colitogenic cytokine IFN- γ .

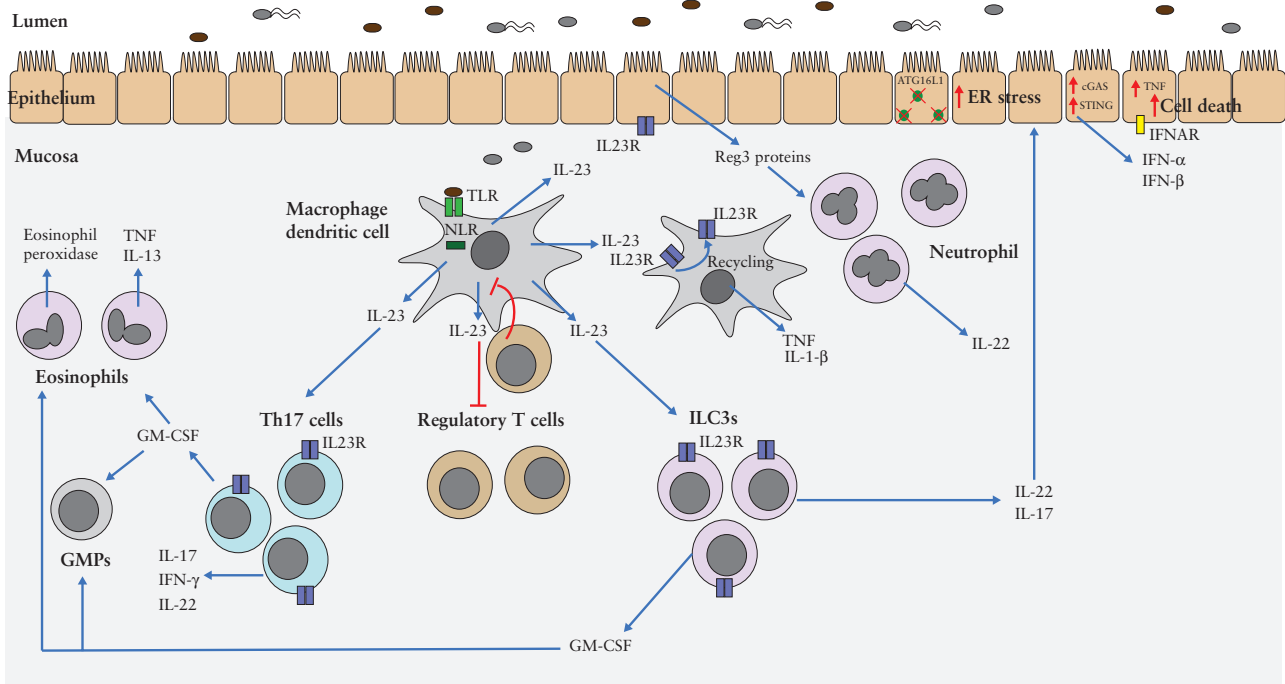


Figure 3. IL-23 promotes intestinal inflammation through pleiotropic effects on innate and adaptive immune cells. Figure depicts the diverse downstream immunological effects of IL-23 produced by macrophages and dendritic cells in response to microbial stimulation. IL-23 enhances the induction and survival of Th17 cells, releasing the IL-17, IFN- γ , IL-22 and GM-CSF cytokines, the last of these promoting accumulation of granulocyte-monocyte progenitor cells [GMPs] and activated eosinophils in the intestine. IL-23 inhibits the induction of regulatory T cells, and orchestrates the production of cytokines such as IL-17, IL-22 and GM-CSF from group 3 innate lymphoid cells [ILC3s]. Autocrine effects of IL-23 on macrophages including pro-inflammatory cytokine production and IL-23R recycling have also been reported. IL-23 additionally induces the production of neutrophil chemoattractant Reg proteins by intestinal epithelial cells, in turn serving as an additional source of IL-22. IL-22 exerts dichotomous effects in intestinal inflammation, including induction of ER stress in the intestinal epithelium and promotion of excessive activation of the cGAS-STING pathway in the absence of ATG16L1, leading to type I interferon production and cell death.

T cells has been identified in inflamed mucosa of both CD and UC patients compared to non-inflamed controls.^{80,129} Additional important sources of IFN γ in the inflamed mucosa may include ILCs and NK cells, which are also responsive to IL-23.¹³⁰ IFN γ is itself well established to promote colitis

and its neutralization is protective in experimental models.¹³¹ Interestingly, fontolizumab, a humanized monoclonal anti-IFN γ antibody, did indicate possible benefit in moderate to severe CD compared to placebo in a multicentre phase 2 study, albeit not demonstrating any statistically significant

difference in the study primary endpoint of clinical remission after 28 days of treatment.¹³² Inhibition of IFN γ has not to date been explored in UC and this could merit consideration.

5.2. Regulatory T cells

Regulatory CD4⁺ T cells [Tregs] have an important role in the promotion of tolerance and restraint of immune responses in the intestine.¹³³ These cells develop in the thymus, but they can also be induced from peripheral naïve T cells in the appropriate context.¹³³ Tregs are characterized by expression of the transcription factor Forkhead box P3 [FoxP3], high cell surface CD25, and production of TGF β and the anti-inflammatory cytokines IL-10 and IL-35.^{133,134} Experimental models support a suppressive effect of these cells in the development of colitis. In the T cell transfer model, co-transfer of CD4⁺ CD45RB^{low} cells, which are enriched for Tregs, prevented colitis.⁵¹ Furthermore, adoptive transfer of CD4⁺ CD25⁺ Tregs could resolve established colitis in this model.¹³⁵ IL-23 has been proposed to inhibit the induction of FoxP3⁺ CD4⁺ Tregs in the intestine,¹¹⁰ via cell-intrinsic mechanisms [Figure 3].⁵² Tregs can themselves act to suppress IL-23 release from intestinal macrophages via a mechanism that appears unrelated to IL-10 production, instead being dependent on cell contact and expression of latent activation gene-3 [LAG3], and consequentially inhibiting IL-22 production by ILCs as a downstream effect.¹³⁶ This highlights the interdependence and connectedness of different pathways involved in the development of intestinal inflammation.

Genetic mutations affecting Treg function, for example in the *FOXP3* gene, can cause the immune dysregulation, polyendocrinopathy, enteropathy, X linked syndrome [IPEX], which is associated with the development of colitis.¹³⁷ However, the overwhelming majority of patients with IBD do not harbour such mutations, nor have genetic studies strongly implicated genes linked specifically to Treg differentiation or function with polygenic IBD susceptibility.¹ Furthermore, isolated CD4⁺ CD25^{high} Treg cells from IBD patients do not display an intrinsic functional defect in their suppressive capacity *ex vivo*, and FoxP3⁺ Treg cells accumulate in the inflamed IBD mucosa compared to non-inflamed controls.¹³⁸ Nonetheless these Tregs are clearly not able to suppress inflammation in the IBD milieu, potentially due to resistance of effector CD4⁺ T cells to suppression by Tregs,¹³⁹ which might have therapeutic implications. Cellular therapy using antigen [ovalbumin]-specific Tregs has been attempted in an open label study of 20 patients with CD, although many unanswered questions remain, including optimal dosing, timing and antigen specificity of the cells transferred.^{140,141} Low-dose IL-2 therapy, which preferentially expands Tregs over effector T cells, has shown promise in a humanized mouse model of colitis,¹⁴² and early studies to primarily assess safety and tolerability in UC have recently been completed [NCT02200445].

5.3. Innate lymphoid cells and the IL-23–IL-22 axis

IL-23 has emerged as a critical orchestrator of ILC function. ILCs are a family of innate immune cells derived from a common lymphoid progenitor, with absent RAG-dependent rearranged antigen receptors, and subdivided phenotypically on the basis of surface marker expression and functional characteristics.^{143,144} ILC1s secrete high levels of IFN γ and thereby enable the host response to intracellular pathogens,

and ILC2s produce IL-4, IL-5 and IL-13 upon activation to facilitate alternative macrophage activation and tissue repair and remodelling.¹⁴⁵ Contrastingly, ILC3s release high levels of IL-17 and IL-22 in response to IL-23 stimulation,^{146–148} the latter reportedly dependent on both STAT3¹⁴⁹ and STAT5 activation.¹⁵⁰ ILCs responsive to IL-23 are increased in inflamed ileum and colon of CD, but not UC, patients, compared to non-inflamed mucosa from control individuals.¹⁵¹ In experimental colitis induced by *Helicobacter hepaticus* in *Rag*^{-/-} mice, in which typhlocolitis occurs in an IL-23-dependent fashion,⁴⁹ depletion of Thy1^{high} SCA-1⁺ ILCs prevented the development of colitis.¹⁵² Furthermore, depletion of the same ILCs attenuated colitis after agonistic anti-CD40 antibody administration, which corresponded to a significant reduction in IL-22, TNF α and IFN γ secretion from isolated colonic lamina propria cells.¹⁵²

IL-22, a member of the IL-10 family of cytokines, has dichotomous roles in intestinal inflammation. Alongside group 3 ROR γ T⁺ ILCs, importantly IL-22 is also produced by Th17 cells, $\gamma\delta$ T cells and neutrophilic granulocytes in response to IL-23.^{25,26,28,153} On the one hand, IL-22 has apparent protective roles in intestinal inflammation. Loss of IL-22 exacerbates DSS-induced colitis, and T cell-mediated colitis in adoptive transfer models.¹⁵⁴ Furthermore IL-22 is required for optimal host defence against pathogens; mice lacking IL-22 demonstrate increased bacterial burden, impaired epithelial integrity and decreased survival following *Citrobacter rodentium* inoculation.¹⁵⁵ These effects were attributed to induction of antimicrobial protein expression such as Reg3b and Reg3g, members of the secreted C-type lectin protein family.¹⁵⁵ Reg3b is itself a STAT3 target gene and is produced by epithelial cells in response to IL-23, and can serve as a chemoattractant for neutrophils which contribute to IL-22 production in the mucosa.²⁶ IL-22 derived from ILC3s and $\gamma\delta$ T cells has also been reported to protect colonic LGR5⁺ stem cells from malignant transformation, enabling an effective DNA damage response following exposure to carcinogens.¹⁵⁶

In spite of these beneficial effects, an emerging body of evidence suggests that pro-inflammatory effects of IL-22 can exacerbate intestinal inflammation. Neutralization of IL-22 was noted to inhibit the development of anti-CD40 antibody-induced colitis in immunodeficient hosts.²⁴ Furthermore, the spontaneous colitis observed in macrophage-restricted IL-10R α -deficient mice was reported to be contingent on both IL-23 expression by macrophages and IL-22 production, proposed to derive primarily from Th17 cells in this model.¹⁵⁷ IL-22 enhances ER stress in epithelial cells,^{158,159} a process critically intertwined with the development of intestinal inflammation.¹⁴ Genetic deficiency and neutralization of IL-22 in the murine TRUC model of IBD dramatically inhibited the development of colitis, which correlated with diminished levels of ER stress in the mucosa.¹⁵⁸ Notably, autophagy, and specifically the product of the *ATG16L1* gene linked to CD susceptibility, appears to be a crucial regulator of IL-22 signalling and downstream biological responses in the intestinal epithelium.¹⁵⁹ Using small intestinal organoids generated from mice lacking *ATG16L1* expression in IECs, IL-22 was shown to induce ER stress and type I IFN responses, which were pathologically enhanced in the absence of *ATG16L1* as a result of excessive activation of the cGAS-STING pathway.¹⁵⁹ Accordingly, exogenous IL-22 administration *in vivo* exacerbated the spontaneous small intestinal inflammation observed in mice dually deficient in *ATG16L1* and *XBP1* in IECs

[*Atg16l1*^{ΔIEC}/*Xbp1*^{ΔIEC}], and furthermore worsened ileal inflammation in *Atg16l1*^{ΔIEC} mice acutely exposed to low-dose DSS. Inhibition of the IFN α / β receptor ameliorated the ileitis potentiated by IL-22 in the latter, highlighting an important pathogenic role for type I IFN responses in this context.¹⁵⁹

Clearly the observations described above have important therapeutic implications. Although early translation has focused on stimulation of IL-22 signalling using recombinant IL-22 fusion proteins¹⁶⁰ [NCT02749630], IL-22 antagonism could in fact be beneficial in certain contexts or selected patients. Indeed, serum and mucosal levels of IL-22 appear overall increased in IBD,^{158,161} and recent transcriptional profiling furthermore identified an IL-22 responsive gene expression signature as significantly upregulated in the inflamed IBD mucosa.¹⁵⁸ Enrichment for this IL-22 transcriptional signature positively correlated with markers of intestinal inflammation such as faecal calprotectin levels, albeit not differentiating clinical response to p40 blockade.¹⁵⁸ However, it is noteworthy that treatment with p19 neutralizing antibodies reduces serum IL-22 levels.^{63,64} In addition, patients stratified by higher baseline IL-22 level appeared to demonstrate greater benefit from selective p19 inhibition,⁶³ further hinting at the importance of the IL-23–IL-22 axis in shaping the clinical effectiveness of p40 and selective p19 blockade. Furthermore, targeting downstream effects of IL-22, including pharmacological inhibition of ER stress, shown to be efficacious in the TRUC experimental model of IBD,¹⁵⁸ as well as antagonism of augmented type I IFN signalling, could equally represent very promising therapeutic targets in patients with underlying dysfunction of the IL-23–IL-22 axis.

5.4. Myeloid cells and autocrine regulation

Infiltration of the intestinal mucosa by neutrophils is a key feature of experimental colitis and inflamed human IBD lesions, particularly UC.¹⁶² Although neutrophils exert beneficial functions in the clearance of pathogens and resolution of acute inflammation, and indeed monogenic disorders causing defective neutrophil function are associated with the development of CD-like enteritis,^{137,163} neutrophils may perpetuate established intestinal inflammation through the production of pro-inflammatory cytokines, chemokines and matrix metalloproteinases.^{162,164,165} In acute infection and inflammation, IL-23 promotes neutrophil recruitment via both IL-17A-dependent and IL-17A-independent mechanisms dependent on context.^{166,167} IL-23 appears to indirectly promote granulopoiesis,¹⁶⁸ and in homeostasis, efferocytosis of apoptotic neutrophils by DCs may serve to regulate neutrophil production by dampening of IL-23 production as part of a negative feedback loop.¹⁶⁹ In experimental T cell transfer colitis, IL-23 potentiates GM-CSF production by Th17 cells, which in turn enhances extramedullary haematopoiesis and the accumulation of proliferative granulocyte–monocyte progenitor cells in the colon and spleen.¹⁶⁵ Furthermore, GM-CSF enhances the production and activation of eosinophils, which have also been proposed to contribute to tissue damage in colitis through activity of eosinophil peroxidase. As chemical inhibition of eosinophil peroxidase improved murine experimental colitis induced by *Helicobacter hepaticus* and IL-10R blockade, this might represent an interesting target for future development.¹⁷⁰

Alongside their critical role in secreting IL-23, myeloid cells such as macrophages concurrently express the IL-23R on their cell surface at low levels, implying potential autocrine effects. *In vivo* administration of IL-23, but not IL-12, was recognized

to stimulate TNF and IL-1 β expression in murine peritoneal macrophages.¹⁷¹ Concordantly, IL-23 stimulates the release of pro-inflammatory cytokines by human monocyte-derived macrophages, coinciding with induction of IL-23R endocytic recycling and recruitment of the IL-12R β 1, JAK2 and other signalling intermediates to the IL-23R complex.²⁷ Macrophages cultured from donors expressing the protective R381Q IL-23R variant appeared diminished in their capacity for cytokine induction²⁷ and reactive oxygen and nitrogen species generation,¹⁷² and demonstrated reduced IL-23R recycling.²⁷ The extent to which IL-23R recycling in myeloid cells contributes to the development of intestinal inflammation *in vivo* remains to be fully elucidated, as does whether similar mechanisms might operate in cells with correspondingly high IL-23R expression such as ILCs and T cells, in which IL-23 has also been reported to enhance expression of its cognate receptor.⁸⁰

6. Concluding Remarks

The prominence of IL-23 in the pathogenesis of both CD and UC, alongside other autoimmune diseases, is now unassailable. However, the downstream effector mechanisms by which IL-23 drives the manifestation of chronic IBD are a continued matter of debate and investigation, in particular the relative importance of Th1/Th17 cells, ILC3s and myeloid cells, and the colitogenic cytokines IFN γ , IL-22 and IL-17A. Despite the closely intertwined role of IL-23 in distinct autoimmune diseases, it is likely that there are important tissue-specific differences in determining how IL-23 contributes to disease; IL-17A induction potentially has a more deleterious role in the development of ankylosing spondylitis and psoriasis rather than colitis, highlighted by the clinical effectiveness of IL-17A blockade in these conditions but not in IBD. The contribution of IL-23 to inflammatory disease is modified and shaped by distinct environmental factors such as diet, the host microbiome and additional genetic determinants, evident in controlled experimental murine colitis models.^{173,174} These will of course be highly heterogeneous between patients.

Therapies targeting the IL-12 and IL-23 shared p40 subunit are established in current clinical practice for IBD, and recent phase 2 studies support the use of specific p19 blockade in CD and UC. Further careful evaluation of these agents continues to be needed in specific patient groups, such as in pregnancy and children with refractory IBD, although recent small-scale studies do indicate potential safety and efficacy of p40 blockade in the latter.^{175,176} More broadly, whilst evidence from a recent cohort study suggests superiority of p40 inhibition over vedolizumab in refractory CD,¹⁷⁷ randomized controlled trials directly comparing the efficacy of p19 with p40 blockade, and with distinct classes of biologics, will be vital to undertake in the future. Indeed, in psoriasis, specific targeting of p19 with the monoclonal antibody guselkumab was reported to show improved efficacy compared to adalimumab.¹⁷⁸ Identification of patients who may benefit from early use of p19 inhibition and related therapies, integrating key biomarkers that may predict responsiveness such as serum IL-22 with applicable genetic data, may also be a powerful therapeutic strategy.

Funding

Research in Professor Kaser's laboratory has been supported by Wellcome Trust senior investigator awards [106260/Z/14/Z and 222497/Z/21/Z to A.K.]. G.W.S is sup-

ported by an Academy of Medical Sciences starter grant for clinical lecturers [SGL022\1008]. This paper was published as part of a supplement financially supported by AbbVie.

Conflict of Interest

A.K. has provided scientific consultancy for Applied Molecular Transport, AstraZeneca, Boehringer Ingelheim, Galapagos, Genentech, GlaxoSmithKline, Glenmark, Gilead Sciences, Hospira, Janssen, Kintai Therapeutics, Novartis, Pfizer, Roche and VHSquared. G.W.S. does not have any conflicts of interest to declare.

Author Contributions

G.W.S. and A.K. both drafted and approved the final manuscript.

Data Availability

No new data were analysed or generated in support of this review article.

References

- Uhlir HH, Powrie F. Translating immunology into therapeutic concepts for inflammatory bowel disease. *Annu Rev Immunol* 2018;**36**:755–81.
- Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. *Annu Rev Immunol* 2010;**28**:573–621.
- Hugot JP, Chamaillard M, Zouali H, *et al.* Association of *NOD2* leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001;**411**:599–603.
- Ogura Y, Bonen DK, Inohara N, *et al.* A frameshift mutation in *NOD2* associated with susceptibility to Crohn's disease. *Nature* 2001;**411**:603–6.
- Hisamatsu T, Suzuki M, Reinecker HC, *et al.* *CARD15/NOD2* functions as an antibacterial factor in human intestinal epithelial cells. *Gastroenterology* 2003;**124**:993–1000.
- Ramanan D, Tang MS, Bowcutt R, Loke P, Cadwell K. Bacterial sensor *NOD2* prevents inflammation of the small intestine by restricting the expansion of the commensal *Bacteroides vulgatus*. *Immunity* 2014;**41**:311–24.
- Smith AM, Rahman FZ, Hayee B, *et al.* Disordered macrophage cytokine secretion underlies impaired acute inflammation and bacterial clearance in Crohn's disease. *J Exp Med* 2009;**206**:1883–97.
- Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell* 2008;**132**:27–42.
- Cadwell K, Liu JY, Brown SL, *et al.* A key role for autophagy and the autophagy gene *Atg16L1* in mouse and human intestinal Paneth cells. *Nature* 2008;**456**:259–63.
- Saitoh T, Fujita N, Jang MH, *et al.* Loss of the autophagy protein *Atg16L1* enhances endotoxin-induced IL-1 β production. *Nature* 2008;**456**:264–8.
- Adolph TE, Tomczak MF, Niederreiter L, *et al.* Paneth cells as a site of origin for intestinal inflammation. *Nature* 2013;**503**:272–6.
- Hampe J, Franke A, Rosenstiel P, *et al.* A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in *Atg16L1*. *Nat Genet* 2007;**39**:207–11.
- Tschurtschenthaler M, Adolph TE, Ashcroft JW, *et al.* Defective *Atg16L1*-mediated removal of IRE1 α drives Crohn's disease-like ileitis. *J Exp Med* 2017;**214**:401–22.
- Kaser A, Lee AH, Franke A, *et al.* Xbp1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell* 2008;**134**:743–56.
- Jostins L, Ripke S, Weersma RK, *et al.* Host–microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012;**491**:119–24.
- Hanauer SB, Feagan BG, Lichtenstein GR, *et al.* Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002;**359**:1541–9.
- Sandborn WJ, van Assche G, Reinisch W, *et al.* Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2012;**142**:257–65 e1–3.
- Duerr RH, Taylor KD, Brant SR, *et al.* A genome-wide association study identifies *IL23R* as an inflammatory bowel disease gene. *Science* 2006;**314**:1461–3.
- Capon F, Di Meglio P, Szaub J, *et al.* Sequence variants in the genes for the interleukin-23 receptor (*IL23R*) and its ligand (*IL12B*) confer protection against psoriasis. *Hum Genet* 2007;**122**:201–6.
- Wellcome Trust Case Control C. Association scan of 14,500 nsSNPs in four common diseases identifies variants involved in autoimmunity. *Nat Genet* 2007;**39**:1329–37.
- Oppmann 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**:715–25.
- Moschen AR, Tilg H, Raine T. *IL-12*, *IL-23* and *IL-17* in IBD: immunobiology and therapeutic targeting. *Nat Rev Gastroenterol Hepatol* 2019;**16**:185–96.
- Neurath MF. *IL-23* in inflammatory bowel diseases and colon cancer. *Cytokine Growth Factor Rev* 2019;**45**:1–8.
- Eken A, Singh AK, Treuting PM, Oukka M. *IL-23R*+ innate lymphoid cells induce colitis via interleukin-22-dependent mechanism. *Mucosal Immunol* 2014;**7**:143–54.
- Sutton CE, Lalor SJ, Sweeney CM, *et al.* Interleukin-1 and *IL-23* induce innate *IL-17* production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* 2009;**31**:331–41.
- Aden K, Rehman A, Falk-Paulsen M, *et al.* Epithelial *IL-23R* signaling licenses protective *IL-22* responses in intestinal inflammation. *Cell Rep* 2016;**16**:2208–18.
- Sun R, Hedl M, Abraham C. *IL23* induces *IL23R* recycling and amplifies innate receptor-induced signalling and cytokines in human macrophages, and the IBD-protective *IL23R* R381Q variant modulates these outcomes. *Gut* 2020;**69**:264–73.
- 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**:12768–73.
- Rachitskaya AV, Hansen AM, Horai R, *et al.* Cutting edge: Nkt cells constitutively express *IL-23* receptor and *ROR γ* and rapidly produce *IL-17* upon receptor ligation in an *IL-6*-independent fashion. *J Immunol* 2008;**180**:5167–71.
- Parham C, Chirica M, Timans J, *et al.* A receptor for the heterodimeric cytokine *IL-23* is composed of *IL-12R β 1* and a novel cytokine receptor subunit, *IL-23R*. *J Immunol* 2002;**168**:5699–708.
- Bloch Y, Bouchareychas L, Merceron R, *et al.* Structural activation of pro-inflammatory human cytokine *IL-23* by cognate *IL-23* receptor enables recruitment of the shared receptor *IL-12R β 1*. *Immunity* 2018;**48**:45–58 e6.
- Glassman CR, Mathiharan YK, Jude KM, *et al.* Structural basis for *IL-12* and *IL-23* receptor sharing reveals a gateway for shaping actions on T versus NK cells. *Cell* 2021;**184**:983–99 e24.
- Bacon CM, McVicar DW, Ortaldo JR, *et al.* Interleukin 12 (*IL-12*) induces tyrosine phosphorylation of *JAK2* and *TYK2*: differential use of janus family tyrosine kinases by *IL-2* and *IL-12*. *J Exp Med* 1995;**181**:399–404.
- Mangan PR, Harrington LE, O'Quinn DB, *et al.* Transforming growth factor- β induces development of the T(h)17 lineage. *Nature* 2006;**441**:231–4.
- Cooper AM, Kipnis A, Turner J, *et al.* Mice lacking bioactive *IL-12* can generate protective, antigen-specific cellular responses to mycobacterial infection only if the *IL-12 p40* subunit is present. *J Immunol* 2002;**168**:1322–7.

36. Happel KI, Dubin PJ, Zheng M, *et al.* Divergent roles of IL-23 and IL-12 in host defense against *Klebsiella pneumoniae*. *J Exp Med* 2005;202:761–9.
37. Zhang F, Liu H, Chen S, *et al.* Identification of two new loci at IL23R and RAB32 that influence susceptibility to leprosy. *Nat Genet* 2011;43:1247–51.
38. Altare F, Lammis D, Revy P, *et al.* Inherited interleukin 12 deficiency in a child with Bacille Calmette-Guerin and *Salmonella enteritidis* disseminated infection. *J Clin Invest* 1998;102:2035–40.
39. Fisher SA, Tremelling M, Anderson CA, *et al.* Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. *Nat Genet* 2008;40:710–2.
40. Pidasheva S, Trifari S, Phillips A, *et al.* Functional studies on the IBD susceptibility gene IL23R implicate reduced receptor function in the protective genetic variant R381q. *PLoS One* 2011;6:e25038.
41. Sivanesan D, Beauchamp C, Quinou C, *et al.* IL23R (interleukin 23 receptor) variants protective against inflammatory bowel diseases (IBD) display loss of function due to impaired protein stability and intracellular trafficking. *J Biol Chem* 2016;291:8673–85.
42. Rivas MA, Beaudoin M, Gardet A, *et al.* Deep resequencing of GWAS loci identifies independent rare variants associated with inflammatory bowel disease. *Nat Genet* 2011;43:1066–73.
43. 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;43:43–7.
44. 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;42:1118–25.
45. Dendrou CA, Cortes A, Shipman L, *et al.* Resolving TYK2 locus genotype-to-phenotype differences in autoimmunity. *Sci Transl Med* 2016;8:363ra149.
46. Velazquez L, Fellous M, Stark GR, Pellegrini S. A protein tyrosine kinase in the interferon alpha/beta signaling pathway. *Cell* 1992;70:313–22.
47. Cunninghame Graham DS, Morris DL, Bhangale TR, *et al.* Association of NCF2, IKZF1, IRF8, IFIH1, and TYK2 with systemic lupus erythematosus. *PLoS Genet* 2011;7:e1002341.
48. Mero IL, Lorentzen AR, Ban M, *et al.* A rare variant of the TYK2 gene is confirmed to be associated with multiple sclerosis. *Eur J Hum Genet* 2010;18:502–4.
49. Hue S, Ahern P, Buonocore S, *et al.* Interleukin-23 drives innate and T cell-mediated intestinal inflammation. *J Exp Med* 2006;203:2473–83.
50. Yen D, Cheung J, Scheerens 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;116:1310–6.
51. Powrie F, Leach MW, Mauze S, Caddle LB, Coffman RL. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in c. B-17 SCID mice. *Int Immunol* 1993;5:1461–71.
52. Ahern PP, Schiering C, Buonocore S, *et al.* Interleukin-23 drives intestinal inflammation through direct activity on T cells. *Immunity* 2010;33:279–88.
53. Elson CO, Cong Y, Weaver CT, *et al.* Monoclonal anti-interleukin 23 reverses active colitis in a T cell-mediated model in mice. *Gastroenterology* 2007;132:2359–70.
54. Becker C, Dornhoff H, Neufert C, *et al.* Cutting edge: IL-23 cross-regulates IL-12 production in T cell-dependent experimental colitis. *J Immunol* 2006;177:2760–4.
55. 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.
56. 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;37:674–84.
57. 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;25:309–18.
58. Mannon PJ, Fuss IJ, Mayer L, *et al.* Anti-interleukin-12 antibody for active Crohn's disease. *N Engl J Med* 2004;351:2069–79.
59. Feagan BG, Sandborn WJ, Gasink C, *et al.* Ustekinumab as induction and maintenance therapy for Crohn's disease. *N Engl J Med* 2016;375:1946–60.
60. Rutgeerts P, Gasink C, Chan D, *et al.* Efficacy of ustekinumab for inducing endoscopic healing in patients with Crohn's disease. *Gastroenterology* 2018;155:1045–58.
61. Sands BE, Sandborn WJ, Panaccione R, *et al.* Ustekinumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2019;381:1201–14.
62. Kock K, Pan WJ, Gow JM, *et al.* Preclinical development of AMG 139, a human antibody specifically targeting IL-23. *Br J Pharmacol* 2015;172:159–72.
63. Sands BE, Chen J, Feagan BG, *et al.* Efficacy and safety of MEDI2070, an antibody against interleukin 23, in patients with moderate to severe Crohn's disease: a phase 2a study. *Gastroenterology* 2017;153:77–86 e6.
64. Feagan BG, Sandborn WJ, D'Haens G, *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;389:1699–709.
65. Feagan BG, Panes J, Ferrante M, *et al.* Risankizumab in patients with moderate to severe Crohn's disease: an open-label extension study. *Lancet Gastroenterol Hepatol* 2018;3:671–80.
66. Sandborn WJ, Ferrante M, Bhandari BR, *et al.* Efficacy and safety of mirikizumab in a randomized phase 2 study of patients with ulcerative colitis. *Gastroenterology* 2020;158:537–49 e10.
67. Sands BE, Peyrin-Biroulet L, Kierkus J, *et al.* Efficacy and safety of mirikizumab in a randomized phase 2 study of patients with Crohn's disease. *Gastroenterology* 2022;162:495–508.
68. Sandborn WJ, Su C, Sands BE, *et al.* Tofacitinib as induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2017;376:1723–36.
69. Ghoreschi K, Jesson MI, Li X, *et al.* Modulation of innate and adaptive immune responses by tofacitinib (CP-690,550). *J Immunol* 2011;186:4234–43.
70. Danese S, Peyrin-Biroulet L. Selective tyrosine kinase 2 inhibition for treatment of inflammatory bowel disease: new hope on the rise. *Inflamm Bowel Dis* 2021;27:2023–30.
71. Fensome A, Ambler CM, Arnold E, *et al.* Dual inhibition of tyk2 and jak1 for the treatment of autoimmune diseases: discovery of ((S)-2,2-difluorocyclopropyl)((1 R,5 S)-3-(2-((1-methyl-1 h-pyrazol-4-yl)amino)pyrimidin-4-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (PF-06700841). *J Med Chem* 2018;61:8597–612.
72. Forman SB, Pariser DM, Poulin Y, *et al.* TYK2/JAK1 inhibitor PF-06700841 in patients with plaque psoriasis: phase IIa, randomized, double-blind, placebo-controlled trial. *J Invest Dermatol* 2020;140:2359–70 e5.
73. Wroblewski ST, Moslin R, Lin S, *et al.* Highly selective inhibition of tyrosine kinase 2 (TYK2) for the treatment of autoimmune diseases: discovery of the allosteric inhibitor BMS-986165. *J Med Chem* 2019;62:8973–95.
74. Tokarski JS, Zupa-Fernandez A, Tredup JA, *et al.* Tyrosine kinase 2-mediated signal transduction in T lymphocytes is blocked by pharmacological stabilization of its pseudokinase domain. *J Biol Chem* 2015;290:11061–74.
75. Burke JR, Cheng L, Gillooly KM, *et al.* Autoimmune pathways in mice and humans are blocked by pharmacological stabilization of the TYK2 pseudokinase domain. *Sci Transl Med* 2019;11.
76. 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.
77. 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.
78. Kvedaraitė E, Lourda M, Idestrom M, *et al.* Tissue-infiltrating neutrophils represent the main source of IL-23 in the colon of patients with IBD. *Gut* 2016;65:1632–41.

79. Schmidt C, Giese T, Ludwig B, *et al.* Expression of interleukin-12-related cytokine transcripts in inflammatory bowel disease: elevated interleukin-23p19 and interleukin-27p28 in Crohn's disease but not in ulcerative colitis. *Inflamm Bowel Dis* 2005;11:16–23.
80. Schmitt H, Billmeier U, Dieterich W, *et al.* Expansion of IL-23 receptor bearing TNFR2+ T cells is associated with molecular resistance to anti-TNF therapy in Crohn's disease. *Gut* 2019;68:814–28.
81. 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;118:2269–80.
82. Pascal V, Pozuelo M, Borruel N, *et al.* A microbial signature for Crohn's disease. *Gut* 2017;66:813–22.
83. Sakuraba A, Sato T, Kamada N, *et al.* Th1/Th17 immune response is induced by mesenteric lymph node dendritic cells in Crohn's disease. *Gastroenterology* 2009;137:1736–45.
84. Gorieli S, Neurath MF, Goldman M. How microorganisms tip the balance between interleukin-12 family members. *Nat Rev Immunol* 2008;8:81–6.
85. Re F, Strominger JL. Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells. *J Biol Chem* 2001;276:37692–9.
86. van Beelen AJ, Zelinkova Z, Taanman-Kueter EW, *et al.* Stimulation of the intracellular bacterial sensor NOD2 programs dendritic cells to promote interleukin-17 production in human memory T cells. *Immunity* 2007;27:660–9.
87. Verreck FA, de Boer T, Langenberg DM, *et al.* Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco)bacteria. *Proc Natl Acad Sci U S A* 2004;101:4560–5.
88. Snijders A, Kalinski P, Hilkens CM, Kapsenberg ML. High-level IL-12 production by human dendritic cells requires two signals. *Int Immunol* 1998;10:1593–8.
89. 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;39:521–36.
90. Aschenbrenner D, Quaranta M, Banerjee S, *et al.* Deconvolution of monocyte responses in inflammatory bowel disease reveals an IL-1 cytokine network that regulates IL-23 in genetic and acquired IL-10 resistance. *Gut* 2021;70:1023–36.
91. Thomas MG, Bayliss C, Bond S, *et al.* Trial summary and protocol for a phase II randomised placebo-controlled double-blinded trial of interleukin 1 blockade in acute severe colitis: the IASO trial. *BMJ Open* 2019;9:e023765.
92. Celic C, Linden J. Purinergic regulation of the immune system. *Nat Rev Immunol* 2016;16:177–92.
93. Schnurr M, Toy T, Shin A, *et al.* Extracellular nucleotide signaling by p2 receptors inhibits IL-12 and enhances IL-23 expression in human dendritic cells: a novel role for the cAMP pathway. *Blood* 2005;105:1582–9.
94. Mogilenko DA, Haas JT, L'Homme L, *et al.* Metabolic and innate immune cues merge into a specific inflammatory response via the UPR. *Cell* 2019;177:1201–16 e19.
95. Cader MZ, Boroviak K, Zhang Q, *et al.* C13orf31 (FAMIN) is a central regulator of immunometabolic function. *Nat Immunol* 2016;17:1046–56.
96. Goodman RP, Markhard AL, Shah H, *et al.* Hepatic NADH reductive stress underlies common variation in metabolic traits. *Nature* 2020;583:122–6.
97. Cader MZ, de Almeida Rodrigues RP, West JA, *et al.* FAMIN is a multifunctional purine enzyme enabling the purine nucleotide cycle. *Cell* 2020;180:278–95 e23.
98. Harrington LE, Hatton RD, Mangan PR, *et al.* Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005;6:1123–32.
99. Tesmer LA, Lundy SK, Sarkar S, Fox DA. Th17 cells in human disease. *Immunol Rev* 2008;223:87–113.
100. Ivanov II, McKenzie BS, Zhou L, *et al.* The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 2006;126:1121–33.
101. Jiang W, Su J, Zhang X, *et al.* Elevated levels of Th17 cells and Th17-related cytokines are associated with disease activity in patients with inflammatory bowel disease. *Inflamm Res* 2014;63:943–50.
102. Ivanov II, Atarashi K, Manel N, *et al.* Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009;139:485–98.
103. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 2006;24:179–89.
104. Zhou L, Lopes JE, Chong MM, *et al.* TGF-beta-induced FOXP3 inhibits T(h)17 cell differentiation by antagonizing RORgammat function. *Nature* 2008;453:236–40.
105. Zhou L, Ivanov II, Spolski 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;8:967–74.
106. Di Meglio P, Di Cesare A, Lagner 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:e17160.
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;108:9560–5.
108. Di Meglio P, Villanova F, Napolitano L, *et al.* The IL23R A/Gln381 allele promotes IL-23 unresponsiveness in human memory T-helper 17 cells and impairs Th17 responses in psoriasis patients. *J Invest Dermatol* 2013;133:2381–9.
109. Nakae S, Nambu A, Sudo K, Iwakura Y. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J Immunol* 2003;171:6173–7.
110. Izcue A, Hue S, Buonocore S, *et al.* Interleukin-23 restrains regulatory T cell activity to drive T cell-dependent colitis. *Immunity* 2008;28:559–70.
111. Noguchi D, Wakita D, Tajima M, *et al.* Blocking of IL-6 signaling pathway prevents CD4+ T cell-mediated colitis in a T(h)17-independent manner. *Int Immunol* 2007;19:1431–40.
112. O'Connor W, Jr, Kamanaka M, Booth CJ, *et al.* A protective function for interleukin 17a in T cell-mediated intestinal inflammation. *Nat Immunol* 2009;10:603–9.
113. Lee JS, Tato CM, Joyce-Shaikh B, *et al.* Interleukin-23-independent IL-17 production regulates intestinal epithelial permeability. *Immunity* 2015;43:727–38.
114. Maxwell JR, Zhang Y, Brown WA, *et al.* Differential roles for interleukin-23 and interleukin-17 in intestinal immunoregulation. *Immunity* 2015;43:739–50.
115. Ito R, Kita M, Shin-Ya M, *et al.* Involvement of IL-17a in the pathogenesis of DSS-induced colitis in mice. *Biochem Biophys Res Commun* 2008;377:12–6.
116. Zhang Z, Zheng M, Bindas J, Schwarzenberger P, Kolls JK. Critical role of IL-17 receptor signaling in acute TNBS-induced colitis. *Inflamm Bowel Dis* 2006;12:382–8.
117. Ogawa A, Andoh A, Araki Y, Bamba T, Fujiyama Y. Neutralization of interleukin-17 aggravates dextran sulfate sodium-induced colitis in mice. *Clin Immunol* 2004;110:55–62.
118. Langley RG, Elewski BE, Lebwohl M, *et al.* Secukinumab in plaque psoriasis—results of two phase 3 trials. *N Engl J Med* 2014;371:326–38.
119. Hueber W, Sands BE, Lewitzky S, *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;61:1693–700.
120. Targan SR, Feagan B, Vermeire S, *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;111:1599–607.
121. Liang SC, Tan XY, Luxenberg DP, *et al.* Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 2006;203:2271–9.

122. El-Behi M, Ciric B, Dai H, *et al.* The encephalitogenicity of T(h)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. *Nat Immunol* 2011;**12**:568–75.
123. Peters A, Lee Y, Kuchroo VK. The many faces of Th17 cells. *Curr Opin Immunol* 2011;**23**:702–6.
124. Schmitt H, Neurath MF, Atreya R. Role of the IL23/IL17 pathway in Crohn's disease. *Front Immunol* 2021;**12**:622934.
125. Bending D, De la Pena H, Veldhoen M, *et al.* Highly purified Th17 cells from BDC2.5NOD mice convert into Th1-like cells in NOD/SCID recipient mice. *J Clin Invest* 2009;**119**:565–72.
126. Hirota K, Duarte JH, Veldhoen M, *et al.* Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat Immunol* 2011;**12**:255–63.
127. Lee YK, Turner H, Maynard CL, *et al.* Late developmental plasticity in the T helper 17 lineage. *Immunity* 2009;**30**:92–107.
128. 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. *Proc Natl Acad Sci U S A* 2015;**112**:7061–6.
129. Globig AM, Hennecke N, Martin B, *et al.* Comprehensive intestinal T helper cell profiling reveals specific accumulation of IFN-gamma+IL-17+ coproducing CD4+ T cells in active inflammatory bowel disease. *Inflamm Bowel Dis* 2014;**20**:2321–9.
130. 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**:882–92, 92 e1–3.
131. Powrie F, Leach MW, Mauze S, *et al.* Inhibition of Th1 responses prevents inflammatory bowel disease in SCID mice reconstituted with CD45RBhi CD4+ T cells. *Immunity* 1994;**1**:553–62.
132. Hommes DW, Mikhajlova TL, Stoinov S, *et al.* Fontolizumab, a humanized anti-interferon gamma antibody, demonstrates safety and clinical activity in patients with moderate to severe Crohn's disease. *Gut* 2006;**55**:1131–7.
133. Izcue A, Coombes JL, Powrie F. Regulatory lymphocytes and intestinal inflammation. *Annu Rev Immunol* 2009;**27**:313–38.
134. Collison LW, Workman CJ, Kuo TT, *et al.* The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 2007;**450**:566–9.
135. Mottet C, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. *J Immunol* 2003;**170**:3939–43.
136. Bauche D, Joyce-Shaikh B, Jain R, *et al.* LAG3(+) regulatory T cells restrain interleukin-23-producing CX3CR1(+) gut-resident macrophages during group 3 innate lymphoid cell-driven colitis. *Immunity* 2018;**49**:342–52 e5.
137. Uhlig HH. Monogenic diseases associated with intestinal inflammation: implications for the understanding of inflammatory bowel disease. *Gut* 2013;**62**:1795–805.
138. Maul J, Loddenkemper C, Mundt P, *et al.* Peripheral and intestinal regulatory CD4+ CD25(high) T cells in inflammatory bowel disease. *Gastroenterology* 2005;**128**:1868–78.
139. Fantini MC, Rizzo A, Fina D, *et al.* SMAD7 controls resistance of colitogenic T cells to regulatory T cell-mediated suppression. *Gastroenterology* 2009;**136**:1308–16, e1–3.
140. Desreumaux P, Foussat A, Allez M, *et al.* Safety and efficacy of antigen-specific regulatory T-cell therapy for patients with refractory Crohn's disease. *Gastroenterology* 2012;**143**:1207–17 e2.
141. Clough JN, Omer OS, Tasker S, Lord GM, Irving PM. Regulatory T-cell therapy in Crohn's disease: challenges and advances. *Gut* 2020;**69**:942–52.
142. Goettel JA, Kotlarz D, Emani R, *et al.* Low-dose interleukin-2 ameliorates colitis in a preclinical humanized mouse model. *Cell Mol Gastroenterol Hepatol* 2019;**8**:193–5.
143. Spits H, Artis D, Colonna M, *et al.* Innate lymphoid cells—a proposal for uniform nomenclature. *Nat Rev Immunol* 2013;**13**:145–9.
144. Cader MZ, Kaser A. Recent advances in inflammatory bowel disease: mucosal immune cells in intestinal inflammation. *Gut* 2013;**62**:1653–64.
145. Eberl G, Colonna M, Di Santo JP, McKenzie AN. Innate lymphoid cells. Innate lymphoid cells: a new paradigm in immunology. *Science* 2015;**348**:aaa6566.
146. Takatori H, Kanno Y, Watford WT, *et al.* Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22. *J Exp Med* 2009;**206**:35–41.
147. 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**:722–5.
148. Longman RS, Diehl GE, Victorio DA, *et al.* CX(3)CR1(+) mononuclear phagocytes support colitis-associated innate lymphoid cell production of IL-22. *J Exp Med* 2014;**211**:1571–83.
149. Guo X, Qiu J, Tu T, *et al.* Induction of innate lymphoid cell-derived interleukin-22 by the transcription factor STAT3 mediates protection against intestinal infection. *Immunity* 2014;**40**:25–39.
150. Bauche D, Joyce-Shaikh B, Fong J, *et al.* IL-23 and IL-2 activation of STAT5 is required for optimal IL-22 production in ILC3s during colitis. *Sci Immunol* 2020;**5**.
151. 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**:1127–33.
152. Buonocore S, Ahern PP, Uhlig HH, *et al.* Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature* 2010;**464**:1371–5.
153. Keir M, Yi Y, Lu T, Ghilardi N. The role of IL-22 in intestinal health and disease. *J Exp Med* 2020;**217**:e20192195.
154. Zenewicz LA, Yancopoulos GD, Valenzuela DM, *et al.* Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity* 2008;**29**:947–57.
155. Zheng Y, Valdez PA, Danilenko DM, *et al.* Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat Med* 2008;**14**:282–9.
156. Gronke K, Hernandez PP, Zimmermann J, *et al.* Interleukin-22 protects intestinal stem cells against genotoxic stress. *Nature* 2019;**566**:249–53.
157. Bernshtein B, Curato C, Ioannou M, *et al.* IL-23-producing IL-10Ralpha-deficient gut macrophages elicit an IL-22-driven proinflammatory epithelial cell response. *Sci Immunol* 2019;**4**.
158. Powell N, Pantazi E, Pavlidis P, *et al.* Interleukin-22 orchestrates a pathological endoplasmic reticulum stress response transcriptional programme in colonic epithelial cells. *Gut* 2020;**69**:578–90.
159. Aden K, Tran F, Ito G, *et al.* ATG16L1 orchestrates interleukin-22 signaling in the intestinal epithelium via cGAS-STING. *J Exp Med* 2018;**215**:2868–86.
160. Stefanich EG, Rae J, Sukumaran S, *et al.* Pre-clinical and translational pharmacology of a human interleukin-22 IGG fusion protein for potential treatment of infectious or inflammatory diseases. *Biochem Pharmacol* 2018;**152**:224–35.
161. Brand S, Beigel F, Olszak T, *et al.* IL-22 is increased in active Crohn's disease and promotes proinflammatory gene expression and intestinal epithelial cell migration. *Am J Physiol Gastrointest Liver Physiol* 2006;**290**:G827–38.
162. Fournier BM, Parkos CA. The role of neutrophils during intestinal inflammation. *Mucosal Immunol* 2012;**5**:354–66.
163. Rahman FZ, Marks DJ, Hayee BH, *et al.* Phagocyte dysfunction and inflammatory bowel disease. *Inflamm Bowel Dis* 2008;**14**:1443–52.
164. Nathan C. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol* 2006;**6**:173–82.
165. Griseri T, McKenzie BS, Schiering C, Powrie F. Dysregulated hematopoietic stem and progenitor cell activity promotes interleukin-23-driven chronic intestinal inflammation. *Immunity* 2012;**37**:1116–29.
166. McDermott AJ, Falkowski NR, McDonald RA, *et al.* Interleukin-23 (IL-23), independent of IL-17 and IL-22, drives neutrophil recruitment and innate inflammation during *Clostridium difficile* colitis in mice. *Immunology* 2016;**147**:114–24.
167. Wu Q, Martin RJ, Rino JG, *et al.* IL-23-dependent IL-17 production is essential in neutrophil recruitment and activity in mouse lung defense against respiratory *Mycoplasma pneumoniae* infection. *Microbes Infect* 2007;**9**:78–86.

168. Smith E, Zarbock A, Stark MA, *et al.* IL-23 is required for neutrophil homeostasis in normal and neutrophilic mice. *J Immunol* 2007;**179**:8274–9.
169. Stark MA, Huo Y, Burcin TL, *et al.* Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. *Immunity* 2005;**22**:285–94.
170. Griseri T, Arnold IC, Pearson C, *et al.* Granulocyte macrophage colony-stimulating factor-activated eosinophils promote interleukin-23 driven chronic colitis. *Immunity* 2015;**43**:187–99.
171. Cua DJ, Sherlock J, Chen Y, *et al.* Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 2003;**421**:744–8.
172. Sun R, Abraham C. IL23 promotes antimicrobial pathways in human macrophages, which are reduced with the IBD-protective IL23R R381Q variant. *Cell Mol Gastroenterol Hepatol* 2020;**10**:673–97.
173. Chen L, He Z, Iuga AC, *et al.* Diet modifies colonic microbiota and CD4⁺ T-cell repertoire to induce flares of colitis in mice with myeloid-cell expression of interleukin 23. *Gastroenterology* 2018;**155**:1177–91 e16.
174. He Z, Chen L, Catalan-Dibene J, *et al.* Food colorants metabolized by commensal bacteria promote colitis in mice with dysregulated expression of interleukin-23. *Cell Metab* 2021;**33**:1358–71 e5.
175. Takeuchi I, Arai K, Kyodo R, *et al.* Ustekinumab for children and adolescents with inflammatory bowel disease at a tertiary children's hospital in Japan. *J Gastroenterol Hepatol* 2021;**36**:125–30.
176. Rosh JR, Turner D, Griffiths A, *et al.* Ustekinumab in paediatric patients with moderately to severely active Crohn's disease: pharmacokinetics, safety, and efficacy results from UNISTAR, a phase 1 study. *J Crohns Colitis* 2021;**15**:1931–42.
177. Biemans VBC, van der Woude CJ, Dijkstra G, *et al.* Ustekinumab is associated with superior effectiveness outcomes compared to vedolizumab in Crohn's disease patients with prior failure to anti-TNF treatment. *Aliment Pharmacol Ther* 2020;**52**:123–34.
178. Blauvelt A, Papp KA, Griffiths CE, *et al.* Efficacy and safety of guselkumab, an anti-interleukin-23 monoclonal antibody, compared with adalimumab for the continuous treatment of patients with moderate to severe psoriasis: results from the phase III, double-blinded, placebo- and active comparator-controlled VOYAGE 1 trial. *J Am Acad Dermatol* 2017;**76**:405–17.