Interleukin-23 in IBD pathogenesis and implications for therapeutic intervention

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Abstract

The 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 *IL-23R* 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 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 inflammatory bowel disease. 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, innate lymphoid, myeloid and intestinal epithelial cells in the context of inflammatory bowel disease pathogenesis. 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.

Keywords:

Interleukin-23, Crohn’s disease, ulcerative colitis

Main text

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 center on an aberrant immune response to microbiota in the gastrointestinal tract.[2] Emerging evidence from a plethora of different functional and genetic studies clearly indicate 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; 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 man, results in an exaggerated UPR favoring inflammation and cell death, characterized by increased expression of the ER stress sensor IRE1α 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 both phenotypes,[15] and the efficacy of therapies such as 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, 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.

IL-23: A pleiotropic cytokine with distinct pro-inflammatory effects

Interleukin-23 (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 disulfide 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 EBI3/p28 and EBI3/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 γδ 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-12Rβ1 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-12Rβ1, thus bridging the IL-23R and IL-12Rβ1 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 IFNγ 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, 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]

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 localised 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 less than 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 IBD pathogenesis, 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 single nucleotide polymorphisms (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 likely causal variant in this region as rs34536443;[45] the protective coding variant resulting 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 interferon 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 corroborates 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, in TNBS-induced colitis, deficiency of IL-23p19 surprisingly enhanced colitis severity.[54] This may relate to enhanced IL-12 production by 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*$^{-/-}$ x *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 dendritic cells (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 versus 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. Biologic 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 seven weeks of treatment, which was associated with a reduction in levels of IL-12, TNFα and IFNγ released by stimulated lamina propria mononuclear cells isolated from colonic biopsies.[58] Subsequently, clear-cut evidence of efficacy of p40 blockade in CD,

in the form of the fully humanised IgG1 monoclonal ustekinumab, was established in the phase 3 UNITI 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 (UNITI-1 cohort) or conventional immunomodulatory therapy (UNITI-2 cohort) failure. The higher clinical response rate corresponded with 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-UNTI 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 versus 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 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 UNITI 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 humanised 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 in this study.[63] Risankizumab is another humanised monoclonal IgG1 developed against the p19 subunit. A multicentre, randomised 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 humanised 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 twelve 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 twelve 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 summarised in Tables 1 and 2).

It could also be envisaged that targeting IL-23R directly, or its downstream signaling 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 placebo[68] 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 IFNα, 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) 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 stabilises 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 interferon 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 demonstrating 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 signaling is influenced by the presence of protective or disease-associated variants in *IL23R* and related genes.

Macrophages and dendritic cells 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 demonstrating 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 $TNFR2^+$ $IL\text{-}23R^+$ $CD4^+$ 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 signaling cascades cumulating 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 complex interplay of factors, some intrinsic to the cell and others related to the surrounding environmental milieu. Firstly, 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 IFNγ.[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 are predicted to enhance IL-23 responses, and indeed 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 harboring *IL-10R* mutations linked to IBD, whereas IL-1R1 blockade was effective in dampening IL-23p19 expression.[90] Thus IL-10 and IL-1β 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 ulcerative colitis,[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 signaling occurring upon bacterial exposure proposed by the authors.[90]

Beyond cytokines, small molecules such as extracellular adenosine nucleotides, known 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 signaling whilst reciprocally suppressing IL-12 responses.[93] Intracellular metabolism within DCs and macrophages appears to provide 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 this 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 enables through its core catalytic activities a purine nucleotide cycle in macrophages.[97] It would therefore be interesting to explore whether additional levels immunometabolic regulation on the IL-23 response might exist, and how these are influenced by host genetic factors. In all, modulation of 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 signaling pathways that they regulate.

Effectors of the IL-23 response

The IL-23-Th17 axis

A major focus of the proinflammatory 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] characterised by expression of the retinoic acid-related orphan receptor γt (RORγt) transcription factor and their signature cytokine IL-17 (also known as IL-17A), alongside others including IL-17F, IL-22 and TNFα. 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 colonisation 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 TGFβ, 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 TGFβ *in vitro* suppresses expression of the transcription factor Forkhead box P3 (FoxP3), reciprocally enhancing RORγt,[104] and furthermore synergistically upregulates expression of the IL-23R.[104,105] This licenses enhanced IL-23R signalling which may further promote IL-17 production (Figure 2 and

Figure 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 upregulated[21]) 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. Firstly, 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 severity of pathology in chemically-induced colitis[113], although 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 sulfate (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, characterised by IFNγ production.[125,126] Restimulation of Th17-polarised $CD4^+$ 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 IFNγ expression over time, when levels of TGFβ 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-$17A^+$ $IFN\gamma^+$ cells in the colon, compared to recipients that received wild type naive cells, as a consequence of diminished proliferation in the intestine.[52] Thus, IFNγ-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 IFNγ expression by the precursor cells.[128] Indeed enrichment of IFNγ$^+$ IL-17A$^+$ CD4$^+$ 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 IFNγ in the inflamed mucosa may include ILCs and NK cells, which are also responsive to IL-23.[130] IFNγ is itself well established to promote colitis and its neutralization protective in experimental models.[131] Interestingly, fontolizumab, a humanized monoclonal anti-IFNγ 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 IFNγ has not to date been explored in UC and this could merit consideration.

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, however 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 TGFβ 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 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 harbor 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).

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 IFNγ 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 STAT3[149] 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 with a significant reduction in IL-22, TNFα and IFNγ 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 RORγt^{+} ILCs, importantly IL-22 is also produced by Th17 cells, γδ 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 demonstrating 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 γδ 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 proinflammatory 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-10Rα-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 signaling 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 interferon 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*$^{\Delta IEC}$ / *Xbp1*$^{\Delta IEC}$), and furthermore worsened ileal inflammation in *Atg16l1*$^{\Delta IEC}$ mice acutely exposed to low dose DSS. Inhibition of the interferon α/β receptor ameliorated the ileitis potentiated by IL-22 in the latter, highlighting an important pathogenic role for type I interferon responses in this context.[158,159]

Clearly the observations described above have important therapeutic implications. Although early translation has focused on stimulation of IL-22 signaling using recombinant IL-22 fusion proteins[160] (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] It is however 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 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 efficacious in the TRUC experimental model of IBD,[158] as well as antagonism of augmented type I interferon

signaling, could equally represent very promising therapeutic targets in patients with underlying dysfunction of the IL-23-IL-22 axis.

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 metalloproteases.[162,164,165] In acute infection and inflammation, IL-23 promotes neutrophil recruitment via both IL-17A-dependent and 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 recognised to stimulate TNF and IL-1β 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-12Rβ1, 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 induction[27] 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 ILCs and T cells, in which IL-23 has also been reported to enhance expression of its cognate receptor.[80]

Concluding Remarks

The prominence of IL-23 in the pathogenesis of both CD and UC, alongside other autoimmune diseases, is now unassailable. The downstream effector mechanisms by which IL-23 drives the manifestation of chronic IBD are however a continued matter of debate and investigation, in particular the relative importance of Th1/Th17 cells, ILC3s and myeloid cells, and the colitogenic cytokines IFNγ, 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 having the 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 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 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 which may predict responsiveness such as serum IL-22 with applicable genetic data, may also be a powerful therapeutic strategy.

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Conflict of Interest statement

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 declare any conflicts of interest.

Data availability statement

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

Author contributions

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

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Figure Legends

Figure 1: Generation of the proinflammatory cytokine IL-23 and its downstream signaling via the IL-23 receptor.

Schematic diagram depicting the production of IL-23 by macrophages and dendritic cells and its downstream effects via the IL-23 receptor. In response to stimulation of Toll-like receptors and NOD-like receptors by microbial agonists, activation of intracellular signaling 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, signaling via specific cell surface receptors. IL-23, a heterodimeric protein comprising of 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, homodimerisation and activation of STAT3. This consequently triggers induction of STAT3 target genes such as *IL17A*, *IL22, IFNG* and *REG3G.*

Figure 2: The IL-23/ Th17 axis.

Naïve $CD4^+$ 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 RORγt and FoxP3 transcription factors respectively, alongside increased IL-23 receptor expression. IL-23 signaling 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 depicting 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, IFNγ, IL-22 and GM-CSF cytokines, the latter promoting accumulation of granulocyte-monocyte progenitor cells (GMPs) and activated eosinophils in the intestine. IL-23 reciprocally 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 proinflammatory 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.

Table 1: Summary of novel therapies targeting IL-23 and the IL-23R signaling pathway in Crohn's disease

Summary of novel therapies targeting IL-23 and its downstream signaling 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).

Table 2: Summary of novel therapies targeting IL-23 and the IL-23R signaling pathway in ulcerative colitis

Summary of novel therapies targeting IL-23 and its downstream signaling 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).

Figure 1

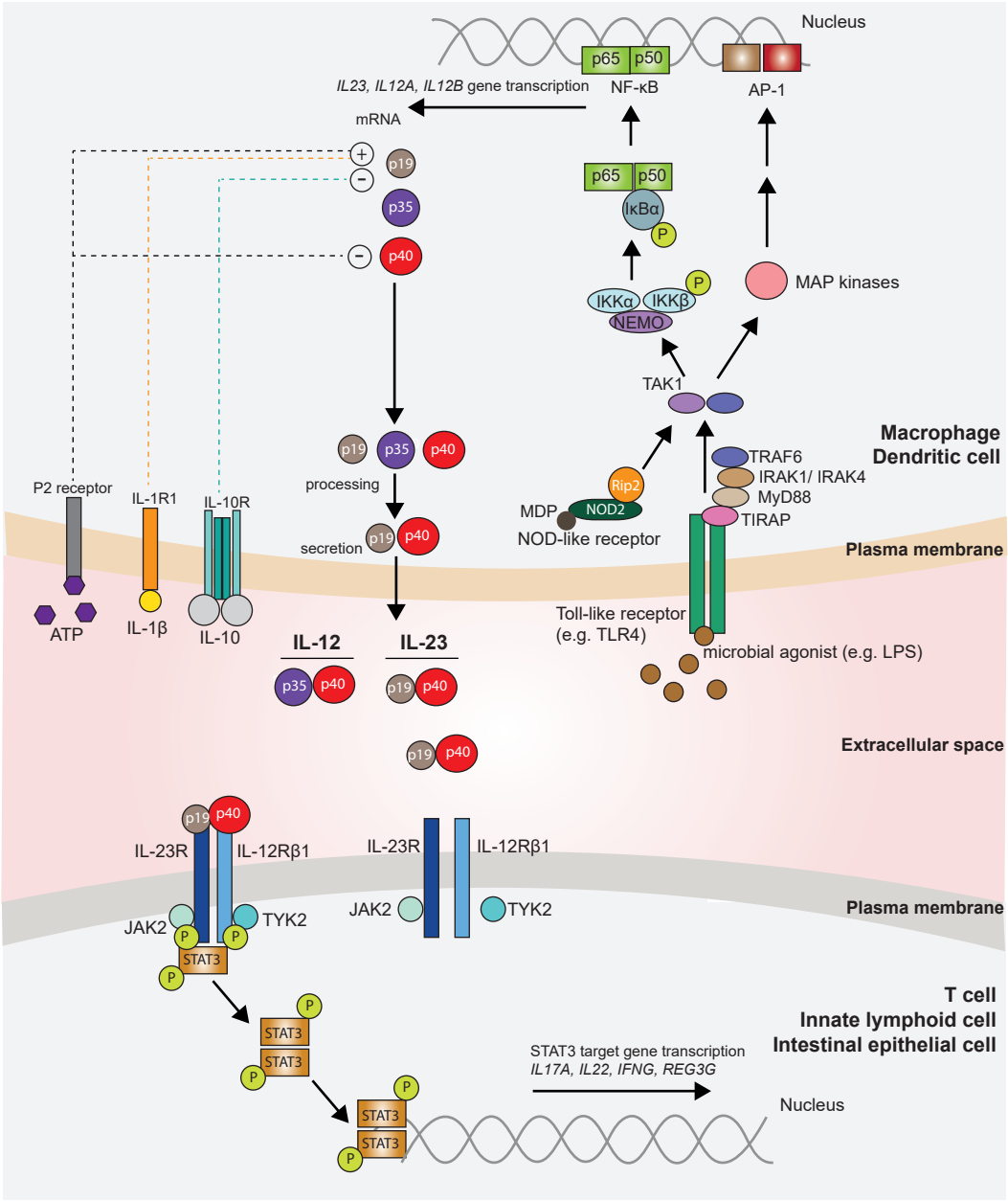

Nucleus
p65
p50
NF-κB
AP-1
IL23, IL12A, IL12B gene transcription
mRNA
p19
p35
p40
p65
p50
IκBα
P
IKKα
IKKβ
NEMO
P
MAP kinases
TAK1
Macrophage
Dendritic cell
processing
secretion
TRAF6
IRAK1/ IRAK4
MyD88
TIRAP
Rip2
MDP
NOD2
NOD-like receptor
P2 receptor
IL-1R1
IL-10R
ATP
IL-1β
IL-10
IL-12
IL-23
Plasma membrane
Toll-like receptor
(e.g. TLR4)
microbial agonist (e.g. LPS)
Extracellular space
IL-23R
IL-12Rβ1
JAK2
TYK2
STAT3
Plasma membrane
T cell
Innate lymphoid cell
Intestinal epithelial cell
STAT3 target gene transcription
IL17A, IL22, IFNG, REG3G
Nucleus

Figure 2

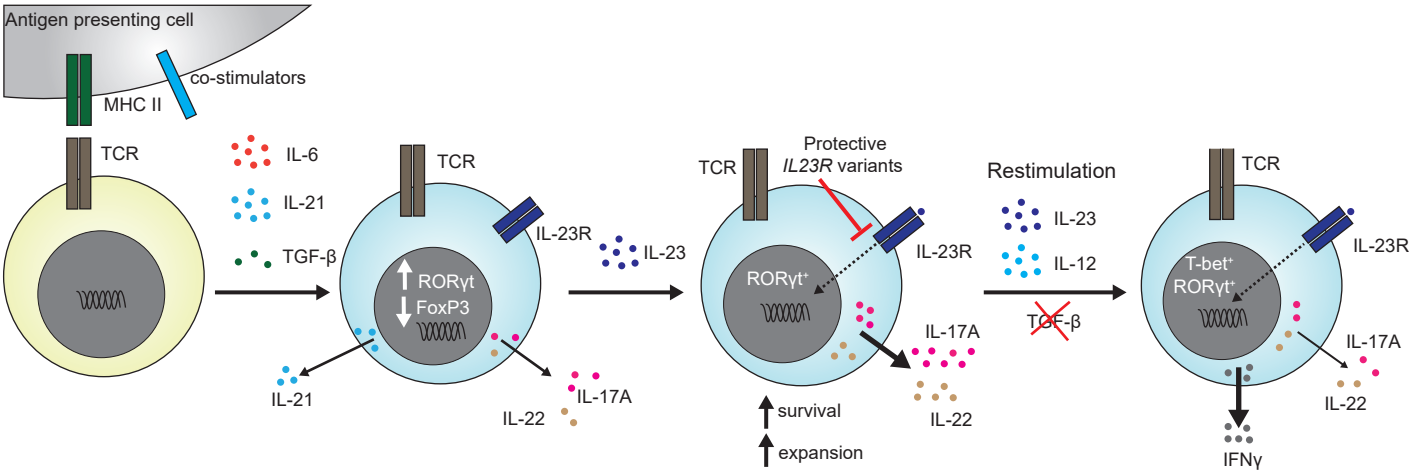
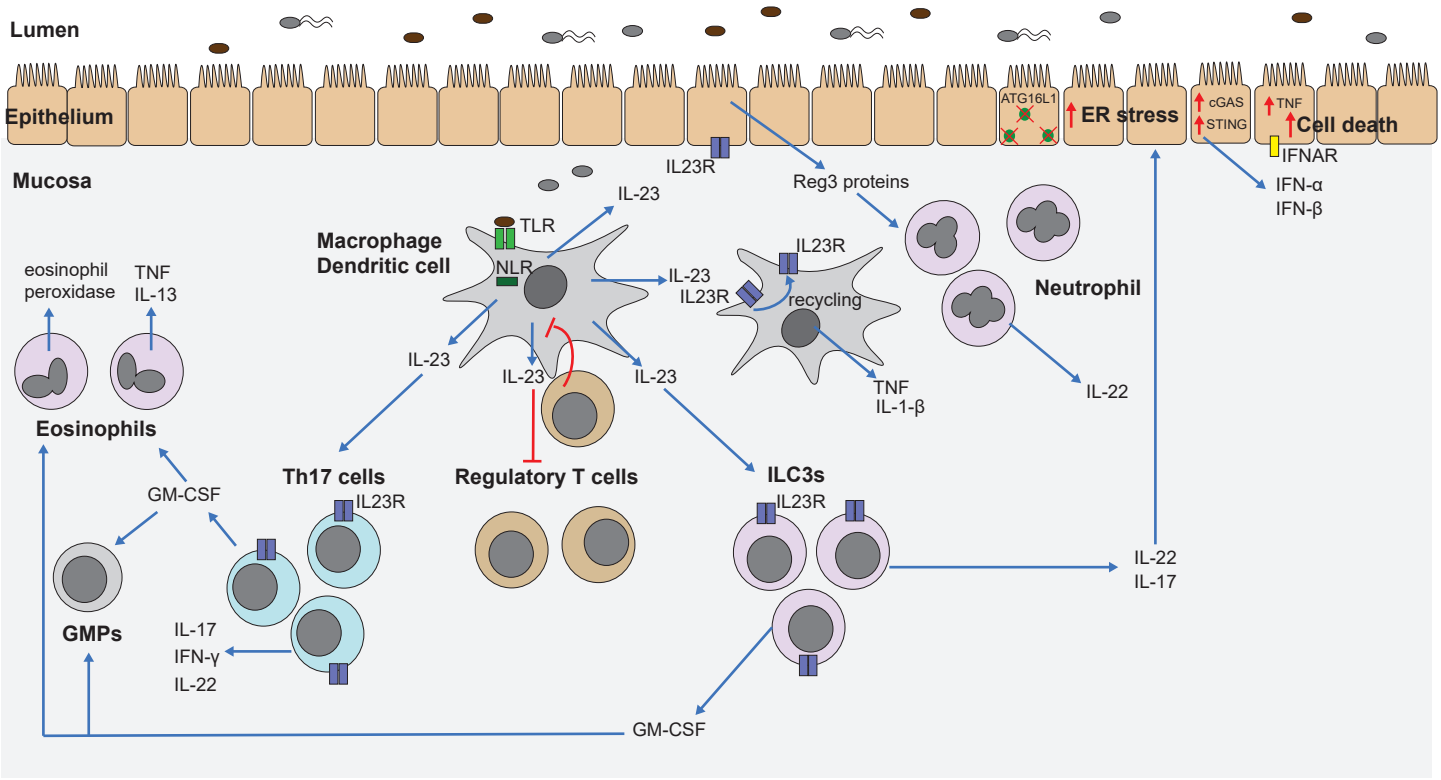

Antigen presenting cell
MHC II
co-stimulators
TCR
IL-6
IL-21
TGF-β
RORγt
FoxP3
IL-23R
IL-23
IL-21
IL-22
IL-17A
Protective
IL23R variants
RORγt+
survival
expansion
Restimulation
IL-12
TGF-β
T-bet+
RORγt+
IFNγ
Naive CD4+ T cell
Th17 cell
IL17+ IFNγ+ T cell

Figure 3


Lumen
Epithelium
ATG16L1
ER stress
cGAS
STING
TNF
Cell death
IFNAR
IFN-α
IFN-β
IL23R
Reg3 proteins
Mucosa
IL-23
TLR
Macrophage
Dendritic cell
NLR
IL-23
IL23R
IL23R
recycling
Neutrophil
TNF
IL-1-β
IL-22
eosinophil
peroxidase
TNF
IL-13
IL-23
IL-23
IL-23
Eosinophils
GM-CSF
Th17 cells
IL23R
Regulatory T cells
ILC3s
IL23R
IL-22
IL-17
GMPs
IL-17
IFN-γ
IL-22
GM-CSF

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	n = 79 Cohort 1 n = 40 Cohort 2 n = 39	Cohort 1: M/F n = 12/28 Mean age: 36.9 years (placebo) 43.1 years (1 mg/kg group) 42.5 years (3 mg/kg group) Cohort 2 M/F n = 14/25 Mean age: 41.0 years (placebo) 39.9 years (1 mg/kg group) 39.8 years (3 mg/kg group)	Primary: safety evaluation of use in Crohn's disease. Secondary: superior clinical response (fall in CDAI score of > 100 at week 7) versus placebo.	Mannon et al. *N Engl J Med* 2004;**351**:2069-79
	Ustekinumab	Phase 3 randomised placebo-controlled trials (UNITI-1, UNITI-2 and IM-UNITI studies)	UNITI-1: n = 749 (Failed anti-TNF) UNITI-2: n = 628 (Failed conventional therapy) IM-UNITI: n = 397	UNITI-1: M/F n = 317/432 Mean age: 37.3 years (placebo) 37.4 years (130 mg group) 37.3 years (6 mg/kg group) UNITI-2: M/F n = 293/335 Mean age: 40.2 years (placebo) 39.1 years (130 mg group) 38.4 years (6 mg/kg group) IM-UNITI: M/F 173/224 39.5 years (placebo) 37.9 years (12 weekly group) 38.6 (8 weekly group)	UNITI-1: Significantly enhanced clinical response (fall in CDAI score of > 100, or CDAI <150) at week 6 versus placebo. Response rate: 34.3% (130mg group), 33.7% (6 mg/kg group) versus 21.5% (placebo). UNITI-2: Significantly improved clinical response rate at week 6 versus placebo. Response rate: 51.7% (130mg group), 55.5% (6 mg/kg group) versus 28.7% (placebo). IM-UNITI: Significantly improved rate of clinical remission (CDAI < 150) at week 44 of treatment versus placebo. Remission rate: 53.1% (8 weekly group), 48.8% (12 weekly group) versus 28.7% (placebo group).	Feagan et al. *N Engl J Med* 2016;375(20):1946-1960
IL-23 p19 subunit	Brazikumab (MEDI2070)	Phase 2a randomised placebo-controlled trial	n = 174	M/F n = 45/74 Mean age: 38.1 years (placebo) 34.9 years (MEDI2070)	Significantly improved clinical response rate (reduction in CDAI score of at least 100, or CDAI <150) after 8 weeks treatment (49.2%) compared to placebo (26.7%).	Sands et al. *Gastroenterology* 2017;153(1):77-86.e6.
	Risankizumab	Phase 2 randomised placebo-controlled trial	n = 121	M/F n = 47/74 Mean age: 36 (placebo) 39 (pooled risankizumab groups)	Significantly higher rate of clinical remission (CDAI <150) in 200mg and 600mg pooled treatment groups (30.5%) after 12 weeks of treatment versus placebo (15.4%).	Feagan et al. *Lancet* 2017;389:1699-709.
	Mirikizumab	Phase 2 randomised placebo-controlled trial (SERENITY)	n = 191	M/F n = 93/98 Mean age: 39.0 (placebo) 38.1 (200mg group) 40.4 (600mg group) 37.7 (1000mg group)	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).	Sands et al. *Gastroenterology* 2022;162:495-508

IL-23 receptor	JNJ-67864238	Phase 2 randomised placebo-controlled trial (PRISM)	n = 48		Difference in CDAI after 12 weeks of treatment compared to baseline. Study terminated early as futility criteria met.	NCT04102111
IL-23 p19 subunit	Risankizumab	Phase 3 randomised 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 randomised placebo and active controlled trial (GALAXI)	Estimated n ~2000		Difference in CDAI after 12 weeks 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 randomised placebo-controlled trial (NCT03395184)	Estimated n ~250		Proportion of patients achieving improvement in endoscopic activity (decrease in 3 or more points in SES-CD).	NCT03395184
TYK2	Deucravacitinib	Phase 2a randomised placebo-controlled trial (LATTICE-CD)	Estimated n ~240		Percentage of patients in clinical remission and percentage demonstrating endoscopic response after 12 weeks treatment.	NCT03599622

Table 1: Summary of novel therapies targeting IL-23 and the IL-23R signaling pathway in Crohn's disease

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 randomised placebo-controlled trials (UNIFI study).	n = 961 (induction study) n = 523 (maintenance study)	Induction trial: M/F n = 582/379 Mean age: 41.2 years (placebo), 42.2 years (130 mg group), 41.7 years (6 mg/kg group) Maintenance trial: M/F n = 297/226 Mean age: 42.0 years (placebo) 40.7 years (12 weekly group) 39.6 years (8 weekly group)	Induction trial: Clinical remission significantly higher after 8 weeks treatment (15.6%, 130mg group and 15.5%, 6mg/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. *N Engl J Med* 2019;381:1201-14
IL-23 p19 subunit	Mirikizumab	Phase 2 randomised placebo-controlled trial	n = 249	M/F n = 149/100 Mean age: 42.6 years (placebo) 41.8 years (50mg), 43.4 years (200mg) 42.4 years (600mg)	Clinical remission after 12 weeks treatment compared to placebo - difference not statistically significant.	Sandborn et al. *Gastroenterology* 2020;158:537-49 e10
IL-23 p19 subunit	Mirikizumab	Phase 3 randomised placebo-controlled trial (LUCENT 1, LUCENT 2)	Estimated ~ 1160 participants (NCT03518086) Estimated ~1044 participants (NCT03524092)		Clinical remission (determined using modified Mayo score) after 12 weeks of treatment (LUCENT 1). Clinical remission after 40 weeks of treatment (LUCENT 2).	NCT03518086; NCT03524092
	Risankizumab	Phase 3 randomised placebo-controlled study	Estimated ~942 participants		Percentage of participants achieving clinical remission at week 12 (determined using adapted Mayo score).	NCT03398135
	Guselkumab	Phase 2b/ 3 randomised placebo-controlled trial (QUASAR)	Estimated ~1000 participants		Clinical response (induction study 1) after 12 weeks treatment Clinical remission (induction study 2). Clinical remission after 44 weeks treatment (maintenance study).	NCT04033445
	Brazikumab	Phase 2 study	Estimated ~256 participants		Clinical remission (defined by Modified Mayo score) after 10 weeks treatment.	NCT03616821

JAK1/ TYK2	Brepocitinib	Phase 2b randomised, placebo-controlled study	Estimated ~319 participants		Total Mayo score of participants after 8 weeks of treatment.	NCT02958865
TYK2	Deucravacitinib	Phase 2 randomised, placebo-controlled trial	Estimated ~131 participants		Clinical remission according to Modified Mayo score following 12 weeks treatment.	NCT03934216

Table 2: Summary of novel therapies targeting IL-23 and the IL-23R signaling pathway in ulcerative colitis.