The IL-23/IL-17 pathway in human chronic inflammatory diseases- new insight from genetics and targeted therapies

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Review

The IL-23/IL-17 pathway in human chronic inflammatory diseases – new insight from genetics and targeted therapies

Running title: The IL-23/IL-17 pathway and inflammatory diseases

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Abstract

Chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, spondyloarthritis and psoriasis cause significant morbidity and are a considerable burden for the patients in terms of pain, impaired function and diminished quality of life, as well as for society, because of the associated high health-care costs, and loss of productivity. Our limited understanding of the pathogenic mechanisms involved in these diseases currently hinders early diagnosis and the development of more specific and effective therapies.

The past years have been marked by considerable progress in our insight of the genetic basis of many diseases. In particular, genome-wide association studies (GWAS) performed with thousands of patients have provided detailed information about the genetic variants associated with a large number of chronic inflammatory diseases. These studies have brought to the forefront many genes linked to signaling pathways that were not previously known to be involved in pathogenesis, pointing to new directions in the study of disease mechanisms. GWAS also provided fundamental evidence for a key role of the immune system in the pathogenesis of these diseases, because many of the identified loci map to genes involved in different immune processes. However, the mechanisms by which disease-associated genetic variants act on disease development and the targeted cell populations remain poorly understood. The challenge of the post-GWAS era is to understand how these variants affect pathogenesis, to allow translation of genetic data into better diagnostics and innovative treatment strategies.

Here, we review recent results that document the importance of the IL-23/IL-17 pathway for the pathogenesis of several chronic inflammatory diseases and summarize data that demonstrate how therapeutic targeting of this pathway can benefit affected patients.

Genome-wide association studies in chronic inflammatory diseases

Chronic inflammatory diseases are a group of clinically heterogeneous, unrelated conditions that share common inflammatory pathways and derive from aberrant immune responses of the human immune system. Chronic inflammatory diseases include more than 100 distinct clinical disorders and their incidence in Western populations has been estimated to be in the range of 5-7%[1]. To obtain insight into the pathogenic mechanisms of these diseases, genome-wide association studies (GWAS) have been performed in several of these disorders, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), type 1 diabetes (T1D), multiple sclerosis (MS), juvenile idiopathic arthritis (JIA), primary biliary cholangitis (PBC), psoriasis (Pso), Crohn's disease (CD), ulcerative colitis (UC), and ankylosing spondylitis (AS). These studies have highlighted disease associations with many loci linked to signaling pathways that were not previously known to be involved in pathogenesis, suggesting new directions in the study of disease mechanisms[2-12]. A substantial number of these loci are shared by several chronic inflammatory diseases, a fact that indicates that several of the pathogenic pathways may be shared among different disease conditions[13].

Because of haplotype structure, GWAS identify large clusters of single nucleotide polymorphisms (SNPs) that are in linkage disequilibrium. Thus, only a small part of the genetic variants identified in GWAS are actually causing disease and it remains a challenge to distinguish the "causal" variants from "neutral" variants that are linked to the first ones. To address this point, Farh et al. have developed an algorithm, Probabilistic Identification of Causal SNPs (PICS), based on a statistical analysis of GWAS data to identify causal variants. Their algorithm identified a single most likely causal variant (>75% probability) at 12% of loci linked to 21 autoimmune diseases[14]. This relatively low number supports the notion that functional studies are necessary to elucidate the biological significance of SNPs linked to autoimmunity, and to correlate disease-associated genetic variants with the effector mechanisms implicated in pathogenesis.

Identification of the IL-23 receptor as a key molecule for several chronic inflammatory diseases

The identification of the gene encoding the receptor for IL-23, *IL23R*, as an inflammatory bowel disease gene has provided the first evidence for the importance of the IL-23 signaling pathway in the pathogenesis of IBD[15]. This GWAS revealed that a nonsynonymous SNP (rs11209026) in the *IL23R* gene is associated with Crohn's disease. The rs11209026 variant causes an amino acid exchange (Arg381Gln) in the cytoplasmic tail of the IL-23R and confers strong protection from the disease[15]. The rs11209026 association was confirmed in a study involving a larger cohort[16] and the same variant was also found to be associated with AS[17] and psoriasis[18, 19]. The same studies also detected additional variants in the *IL23R* gene, and in the 50 kb intergenic region between *IL23R* and the gene encoding the signaling subunit for

IL-12, *IL12RB2*. One of these variants maps to an enhancer element that our laboratory has previously shown to be of critical importance for Th1-specific expression of the *IL12RB2* gene[20] and its functional significance has been further investigated in a more recent study[21]. Further evidence that the IL-23 signaling pathway plays an important role in human chronic inflammatory diseases comes from studies that have identified associations with variants in additional genes encoding critical molecules involved in the IL-23/IL-17 pathway, such as variants in the gene encoding the p40 subunit shared between IL-23 and IL-12 (*IL12B*), which are associated with Crohn's disease[16] and psoriasis[18, 19]; and variants in STAT3, the transcription factor relaying IL-23 signals to the nucleus; TYK2, the kinase associated with the IL-12Rβ1 subunit of the IL-23R and CCR6, a chemokine receptor preferentially expressed on human Th17 cells[22, 23], which are associated with Crohn's disease[16].

IL-23 and IL-17-secreting Th17 cells are key players to promote inflammatory diseases in mice

The identification of IL-23, a new member of the IL-12 family of heterodimeric cytokines[24], has forced a reassessment of the role of Th1 cells in autoimmunity[25]. IL-12 and IL-23 share a common subunit, p40, which is covalently linked to the p35 subunit to form IL-12 or to the p19 subunit to form IL-23[26]. Given the similarities of the two cytokines and the fact that they share a common receptor subunit, IL-12Rβ1[27], it was initially assumed that IL-12 and IL-23 have overlapping functions. However, subsequent studies demonstrated that IL-23, but not IL-12, induced the secretion of IL-17 from activated memory T cells[28]. It was therefore proposed that IL-23 induces the development of a distinct subset of effector $CD4^+$ T cells characterized by the secretion of IL-17 (Th17)[29, 30]. The importance of IL-23 in autoimmune inflammation was recognized by analyzing the susceptibility of IL-12 or IL-23 knockout mice to the development of autoimmune diseases. Mice with a deletion of the IL-23p19 subunit (lacking IL-23), but not mice with a deletion of the IL-12p35 subunit (lacking IL-12) were protected from disease in several experimental models of autoimmunity, such as experimental autoimmune encephalomyelitis (EAE)[30, 31], collagen-induced arthritis (CIA)[32], and inflammatory bowel disease (IBD)[33, 34]. Furthermore, mice treated with neutralizing antibodies against IL-23 or IL-17 were protected from the development of EAE. Importantly, treatment of mice with anti-IL-23 or anti-IL-17 antibodies after disease onset prevented EAE relapse, indicating that therapeutic targeting of Th17 cells could be a promising novel approach to inhibit autoimmune inflammation of the central nervous system (CNS)[35]. Taken together, these findings provided strong evidence that Th17 cells represent a distinct subset of $CD4^+$ T lymphocytes that plays a critical role in chronic inflammation and autoimmunity in mice.

It is, however, important to note that Th17 cells may not be the only T cell subset to promote autoimmune inflammation. Subsequent studies demonstrated that both Th17 and

Th1 cells induce disease in the EAE model[36, 37]. Although the clinical symptoms were similar, these studies revealed distinct cellular infiltrates and histological characteristics induced by Th17 and Th1 cells[36] and suggested that the Th17:Th1 ratio is a critical determinant for brain versus spinal cord inflammation[37].

These findings provided strong evidence that $CD4^+$ T cell populations with distinct functional properties may contribute to autoimmune inflammation and urged for the need of a careful analysis of the relative contributions of Th1, Th17, and possibly additional cell populations in inflammatory disease.

IL-23 and innate IL-17-producing cells in human pathologies

IL-23 is important for the expansion and the functional activity of the Th17 cell subset[38]. However, several studies have also suggested that IL-23 may regulate the function of IL-17-producing innate immune cells, which express the IL-23 receptor (IL-23R) in inflammatory disease. In particular, innate lymphoid cells (ILCs) were shown to drive IL-23-dependent intestinal inflammation in mice[39], and were enriched in the intestine of patients affected by inflammatory bowel disease (IBD)[40]. In addition, a subpopulation of γδ T cells that produces IL-17 contributes to experimental autoimmune encephalomyelitis (EAE) in mice[41]. IL-23R-expressing γδ T cells are also enriched in the peripheral blood of spondyloarthritis (SpA) patients[42], and a direct link between IL-23 and tissue inflammation has been established in a mouse model of SpA[43]. Sherlock et al. demonstrated that IL-23 mediates enthesal inflammation, the hallmark of SpA, by acting on a small population of $CD3^+CD4^-CD8^-IL\text{-}23R^+ROR\gamma t^+$ enthesal resident T cells[43]. The implication of the IL-23/IL-17 axis in this disease is also supported by the finding that at least 6 of the non-MHC loci genetically linked with SpA are associated with genes in this pathway (*RUNX3*, *IL23R*, *IL6R*, *IL1R2*, *IL12B*, *TYK2*)[44]. Taken together, these data suggest that the inflammatory response in SpA may be the result of a complex interplay of different immune cell types and that the IL-23/IL-17 pathway is likely to play a key role in chronic inflammation. Understanding the cellular and molecular mechanisms that regulate this network of innate and adaptive immune responses is therefore of critical importance for the design of rational therapies.

To address this question, our lab and others have investigated the impact of genetic polymorphisms in genes of the IL-23 signaling pathway on the effector functions of $CD4^+$ T cells from SpA patients[45-47]. We have measured the expression levels of Th17 and Th1 cytokines and transcription factors in stimulated $CD4^+$ T cells isolated from SpA patients, and we correlated them with the patients' genotype at loci genetically associated with SpA. We showed that SpA patients carrying risk-associated alleles of genes in the IL23/Th17 pathway expressed the highest levels of genes involved in the differentiation and function of Th17 and Th1 cells, such as *IL17A*, *IL17F*, *RORC*, *IFNG*, *TNF*, and *TBX21*, whereas the presence of protective alleles was associated with low-level expression of these genes. In contrast,

variation at loci genetically linked to SpA, but not associated with the IL-23 pathway (such as *ERAP1* and *ANTXR2*), did not correlate with the expression of Th17 and Th1 genes, suggesting that these SNPs may contribute to SpA pathogenesis through distinct cellular mechanisms. These data showed that genetic variation at multiple loci within the IL-23/Th17 pathway, such as *IL23R*, *IL12B* and *CCR6*, affects $CD4^+$ effector functions in SpA patients. Of note, the effect of genetic variation on $CD4^+$ T cell function could be detected only in activated T cells, but not at steady state, consistent with the context-dependent action of expression quantitative trait loci (eQTL) observed in several studies[48-50]. We also showed that the combinatorial action of multiple SNPs at distinct loci, rather than a single genetic variant, determined the immune cell functions of SpA patients and we have established a hierarchy among the SNPs with respect to their effect on regulating the expression of effector molecules using multivariate analysis. These results demonstrate a link between disease-associated genetic variants and defined functions of immune cell populations involved in the pathogenesis of inflammatory disease.

The tyrosine kinase TYK2: a rheostat implicated in inflammatory and infectious diseases

A large number of GWAS performed over the past years have identified genetic variants at the *TYK2* locus that are associated with RA, SLE, T1D, MS, JIA, PBC, Pso, CD, UC, and AS[3-12]. The genetic association of *TYK2* variants with at least 10 distinct chronic inflammatory diseases suggested that this non-receptor tyrosine kinase plays a central role in the pathogenesis of multiple diseases and also provided further evidence for the observed sharing of genetic risk factors across diseases[13]. However, the machanisms of action of *TYK2* variants in pathology remained unclear.

To address this question, Dendrou et al. have performed comprehensive fine-mapping of the *TYK2* locus with GWAS data from 36,000 patients and almost 20,000 controls[6, 8, 10, 12]. Their analysis revealed three independent genetic associations; a primary association at rs34536443, which is likely the causal variant, a secondary association with rs9797854 as the index SNP and a third association with the rs12720356 index SNP[51]. The rs34536443 minor allele was found to be protective across all the 10 chronic inflammatory diseases investigated, with odds ratios (OR) between 0.54 (Pso) and 0.80 (MS) for heterozygous carriers and 0.1 to 0.3 for individuals carrying both minor alleles. This genetic variant is of particular interest because it leads to the exchange of a conserved proline residue in the TYK2 catalytic domain to an alanine (P1104A, denoted in the following as $TYK2^A$). To define if this amino acid exchange has an effect on signal transduction, the authors studied signaling in response to several cytokines in peripheral blood mononuclear cells (PBMC) obtained from the Oxford BioBank resource (https://www.oxfordbiobank.org.uk/), which allows the recruitment of healthy individuals based on their genotype. TYK2 had initially been identified as a molecule of key importance to relay signals by IFN-α and IFN-β via the type I IFN

receptor[52], resulting in the activation of STAT molecules, in particular STAT1, STAT2 and STAT3. Dendrou et al. showed that IFN-α and IFN-β induced significantly less STAT3 phosphorylation in naïve and memory $CD4^+$ and $CD8^+$ T cells, as well as B cells and monocytes from individuals homozygous for the $TYK2^A$ allele ($TYK2^{A/A}$). STAT3 activation was much less affected in $TYK2^{P/A}$ individuals that express both TYK2 alleles. In addition to type I IFN, TYK2 relays signals by a number of additional cytokines, such as IL-6, IL-10, IL-12, IL-13 and IL-23. STAT-activation in response to IL-6, IL-10 and IL-13 was not diminished in $TYK2^{A/A}$ individuals, indicating that TYK2 catalytic activity was not required to transduce signals elicited by these cytokines. In contrast, both IL-12-induced STAT4 activation and IL-23-induced STAT3 phosphorylation were strongly diminished in memory $CD4^+$ and $CD8^+$ T cells from $TYK2^{A/A}$ individuals, when compared to $TYK2^{P/P}$ carriers[51]. Of note, the effect of the TYK2 P1104A variant on STAT activation was only found in homozygous, but not in heterozygous carriers of this variant, consistent with the observed non-additive effect of the rs34536443 genotype on disease risk (**Figure 1**).

To directly test if the TYK2 P1104A variant could have an impact on the pathogenesis of a chronic inflammatory disease, Dendrou et al. generated knock-in mice carrying the orthologous P1104A amino acid substitution in Tyk2, which in mice is at position 1124 (P1124A). Consistent with the data in human cells, B cells, T cells and monocytes from Tyk2 Ala1124 homozygous mice displayed reduced Stat1-phosphorylation in response to IFN-β when compared to cells from Tyk2 Pro1124 homozygotes[51]. Similarly, Stat4 activation in response to IL-12 and IL-23-induced Stat3 activation were diminished in memory $CD4^+$ and $CD8^+$ T cells from these knock-in mice. Importantly, following immunization with the encephalitogenic myelin oligodendrocyte glycoprotein (MOG) peptide to induce experimental autoimmune encephalomyelitis (EAE), an experimental model of human MS, heterozygous $Tyk2^{A/P}$ mice displayed decreased disease incidence and severity compared to $Tyk2^{P/P}$ wild-type mice. Of note, $Tyk2^{A/A}$ homozygous mice were completely protected against EAE[51]. Cytokine knock-out studies had previously shown that mice with a deletion of the IL-23p19 subunit (and thus lacking IL-23), were protected from EAE[31]. Therefore, the observed protection of mice homozygous for the Tyk2 Ala1124 allele is likely to result from diminished IL-23 signaling. Consistent with this notion, CNS-infiltrating $CD4^+$ T cells from $Tyk2^{A/A}$ mice produced substantially less IFN-γ and IL-17A after immunization with MOG peptide. Together, these experiments present an elegant approach of how the impact of disease-associated genetic variants identified by GWAS can be studied in an experimental disease model.

Previous studies had shown that TYK2-deficiency is associated with susceptibility to tuberculosis and Mendelian susceptibility to mycobacterial disease (MSMD)[53, 54]. To investigate if rs34536443 minor allele homozygotes were more exposed to infectious diseases or malignancies, Dendrou et al. screened health records from the donors of the

Oxford BioBank resource and of more than 100,000 genotyped individuals of European ancestry from the UK. Around 0.2% of Europeans are $TYK2^{A/A}$ homozygotes, however, this study found no evidence of increased infectious disease risk in $TYK2^{A/A}$ individuals[51].

A specific role of the TYK2 P1104A variant in predisposing individuals to infectious disease has recently been re-assessed by the Casanova lab in two cohorts of patients with tuberculosis or MSMD[55]. Previous work by this lab and by others had shown that autosomal recessive deficiencies of *IL12RB1* and *TYK2* are rare monogenic causes of tuberculosis, each found in less than 1/600,000 individuals[53, 54, 56-58]. In contrast, approximately 1/600 individuals of European origin are homozygous for the rs34536443 minor allele[55]. To investigate a potential link between rs34536443 and infectious disease, Boisson-Dupuis et al. have analyzed whole-exome sequencing (WES) data from 454 patients with tuberculosis, 463 patients with MSMD and 5339 controls for whom complete WES data were available. While only 1 individual homozygous for the rs34536443 minor allele was identified in the control cohort, 3 MSMD and 7 tuberculosis patients were homozygous for the rs34536443 variant in the disease cohorts. This strong enrichment ($P = 3.27 \times 10^{-3}$, OR = 23.53 for MSMD and $P = 8.37 \times 10^{-8}$, OR = 89.31 for tuberculosis) suggested that homozygosity for TYK2 P1104 is a genetic cause for tuberculosis and MSMD.

To investigate how the TYK2 P1104A variant increased disease risk for tuberculosis and MSMD, Boisson-Dupuis et al. studied cytokine signaling in TYK2-deficient EBV B and herpes virus saimiri (HVS)-transformed T cell lines. These cells were transduced with retroviruses expressing a wild-type (WT), or P1104A-mutant *TYK2* cDNA. Expression of any of these TYK2 constructs restored TYK2 and scaffolding-dependent expression of the type 1 IFN receptor chain, IFN-αR1 and of IL-12Rβ1. Boisson-Dupuis et al. noted that IFN-α-induced STAT1-activation was not reduced in EBV B and HVS T cells transduced with the P1104A TYK2 variant. Furthermore, IL-12-induced STAT4-activation was similar in HVS-transformed T cells transduced with the WT cDNA or the TYK2 P1104A variant. These data contrast the findings of Dendrou et al. obtained with primary cells from the Oxford BioBank (see above) but the reasons for these discrepancies are currently not known. However, both studies reported that IL-23-induced STAT3-activation was severely diminished in cells expressing TYK2 P1104A[51, 55], pointing to a critical role of TYK2 P1104A and IL-23 signaling in infectious and chronic inflammatory diseases. Boisson-Dupuis also investigated the molecular mechanism by which the TYK2 P1104A variant selectively affected IL-23 signaling. While IL-12 signaling can occur in the presence of only one active kinase (JAK2 or TYK2), the authors showed that IL-23 signaling required a catalytically active TYK2 enzyme. The reason for this may be a different positioning of JAK2 and TYK2 in the IL-12 and IL-23 receptor complexes, which determines the different activation modes of these kinases[55].

To provide further support for the notion that IL-23, but not IL-12 signaling is affected by TYK2 P1104A, Boisson-Dupuis et al. stimulated whole blood or PBMC from homozygous

TYK2$^{A/A}$ patients with BCG in the presence or absence of IL-12 or IL-23. Consistent with the observed IL-12-induced STAT4 activation in HVS-transformed T cells expressing a TYK2 P1104A cDNA, IL-12 increased IFN-γ production in primary cells from patients. In contrast, IL-23 did not enhance IFN-γ secretion in this setting (**Figure 1**).

Although the studies by Dendrou et al. and Boisson-Dupuis et al. did not reach the same conclusions in all points, they provide a remarkably detailed view of the molecular mechanism by which homozygosity for a rather frequent allele provides strong protection against various chronic inflammatory disease, mirrored by high susceptibility to an infectious disease[51, 55]. These two studies showed that TYK2 is a central rheostat controlling susceptibility to infections and autoimmunity, which can be tuned by a single genetic variant. These findings also suggested that TYK2 could be an interesting drug target in chronic inflammatory disease.

Targeting the IL-23/IL-17 pathway, a new therapeutic option for chronic inflammatory diseases

Several clinical trials have been performed over the past years to evaluate if targeting TYK2, IL-23 or IL-17 is beneficial for the treatment of chronic inflammatory diseases.

A recent phase 2 clinical study has tested a small molecule inhibitor of TYK2 in patients with moderate-to-severe psoriasis[59]. This inhibitor (BMS-986165) selectively targets the pseudokinase domain of TYK2 and can be administered orally. A total of 267 patients were randomly assigned to one of six treatment groups (5 distinct oral doses of the drug or placebo) and the intervention period was 12 weeks with an additional 30-day period for safety monitoring. Patients included in this trial were not previously treated with drugs targeting the same pathway, i.e. IL-12, IL-23 or IL-17 inhibitors. The primary endpoint of the trial was a 75% or greater reduction from baseline in the "Psoriasis Area and Severity Index" (PASI) score at week 12. The primary endpoint was achieved by 39% of patients receiving 3 mg of the drug daily and 75% of patients treated with a daily dose of 12 mg of this TYK2 inhibitor. The safety profile of this new drug appeared to be acceptable. While further studies in larger cohorts and over longer durations are needed, this study provided proof-of-concept that TYK2 inhibition is a valid alternative for the treatment of a chronic inflammatory disease[59].

The lymphocytic transcription factor RORγt is necessary for the differentiation and function of Th17 cells[60]. RORγt overexpression increased production from Th17 cells of inflammatory cytokines and chemokines, such as IL-17, IL-22, IL-26, CCR6 and CCL20[60]. Other IL-17 producing lymphocyte subsets have been shown to express RORγt, including subsets of CD8+ T cells, γδ cells, type 3 innate lymphocyte cells (ILC3) and a fraction of iNKT cells. RORγt, however, seems to play different roles in the different cell subsets, as demonstrated by the selective suppression of cytokine production by Th17 cells caused by RORγt inhibition in the

mouse. The functions of intestinal ILC3 were largely spared in this model, suggesting that their protective role on the intestinal epithelium may not be affected by this treatment[61]. Similarly, RORγt blockade decreased IL-22 production from human Th17 cells, but not by IL-17$^+$ γδ T cells or iNKT cells, again supporting differential roles of RORγt for cytokine regulation in adaptive and innate immune cells[62, 63]. The selective action of RORγt, and the preservation of a protective mucosa-associated IL-22 response, may suggest that RORγt blockade could be effective in Crohn's disease, where IL-17 blockade had failed[62].

Because of its role in the differentiation and function of inflammatory lymphocytes, RORγt appeared to be an interesting therapeutic target for the treatment of chronic inflammatory disorders. The majority of RORγt inhibitors have targeted the ligand-binding domain (LBD) of the molecule, blocking the binding of co-activators or promoting the recruitment of co-repressor complexes[64, 65].

The inhibition of RORγt in vitro or in preclinical models impaired Th17 development, and increased resistance to EAE or psoriasis development[65], supporting a potential usefulness of this approach for the treatment of psoriasis and MS in humans.

However, the development of highly specific inhibitors is not trivial, given the conservation of the LBD with other members of the Retinoid-related orphan receptors family, which includes RORα and RORβ. RORγt also shares an identical LBD with the isoform RORγ, which displays a broader tissue expression that includes heart, muscle, liver and kidney, raising the issue of potential multiorgan toxicity of RORγt inhibition. An additional target of RORγt inhibition is the thymus, where this transcription factor is highly expressed in thymocytes at the double-positive (DP) stage. Inhibition of RORγt in a mouse model decreased DP thymocyte survival and resulted in a limited T cell receptor repertoire diversity[66]. Additional concerns raised by the effect of loss of RORγt in humans or in murine models is an increased susceptibility to Candida and mycobacterial infections[67], and the development of lymphomas[68].

All these findings may explain the fact that, to this day, no RORγt inhibitor has yet reached Phase III clinical trials. A Phase II trial in psoriasis of the oral inhibitor VTP-43742 was terminated early due to unspecified safety concerns. Several new compounds (see **Figure 2**) have still been tested in Phase I trials, mostly for psoriasis, with only one compound tested for MS. The most advanced compound is the inverse agonist GSK-2981278 tested for topical use in psoriasis. The results from the Phase II trial have been submitted but are not yet publically available (ClinicalTrials.gov NCT03004846).

The IL-23 cytokine has also been targeted directly for the treatment of chronic inflammatory diseases, and has shown remarkable results for the treatment of psoriasis. The efficacy and safety of ustekinumab, a fully human monoclonal antibody that blocks the p40 subunit of IL-23 and IL-12, were tested in a phase 2 clinical study[69]. A 12 weeks treatment of psoriatic

patients with ustekinumab resulted in a 75% improvement of the PASI (psoriasis area-and-severity index) in up to 80% of patients and a 90% PASI improvement in 50% of patients[69]. Two phase 3 studies confirmed these remarkable results[70, 71], shifting the paradigm for psoriasis treatment from the use of TNF-blockers such as etanercept to the more effective IL-12/IL-23 blockers[72]. While treatment with ustekinumab blocks also IL-12, in addition to IL-23, a monoclonal antibody that neutralizes exclusively IL-23 bioactivity (risankizumab, which binds p19) demonstrated an even higher efficacy in the treatment of psoriasis, supporting the pathogenic role of IL-23 in this disease[73].

Increased levels of the IL-17A cytokine can be detected in psoriatic plaques, and antibodies that neutralize IL-17A (sekukinumab and ixekizumab) or block the IL-17RA (brodalumab), hence inhibiting the activity of several members of the IL-17 family, have been approved for the treatment of psoriasis, and have demonstrated a strong efficacy for the treatment of the disease. Although neither IL-17A inhibitors nor IL-23 blockers result in a cure of psoriasis, these new biologics have revolutionized treatment of this frequent disease (2-3% of the general population), with a remarkable impact on the quality of life of psoriasis patients.

Among the side effects of IL-17 blockade are increased Candida infections, in agreement with the role of IL-17 in protective immunity against fungi[74]. A more concerning side-effect is the increased incidence of IBD and worsening of concurrent IBD pathology[75].

Consistently with these side-effects, blocking IL-17A in Crohn's disease patients was ineffective, and actually associated with higher rates of adverse events compared to placebo[76]. A phase 2 study to evaluate safety and efficacy of brodalumab in patients with moderate-to-severe Crohn's disease had to be terminated early, because of the high number of cases of worsening of Crohn's disease in the treatment groups[77].

The detailed mechanisms for the failure of IL-17A inhibitors in Crohn's disease are not completely understood. Studies in mouse colitis models have provided evidence that IL-17A plays a central role of in protecting the barrier integrity of the intestinal epithelium, despite its potent pro-inflammatory properties[78, 79]. Using the dextran sodium sulfate (DSS) model of colitis, Lee et al. demonstrated that the expression of genes controlling epithelial tight junction integrity was not altered in *Il17a*-deficient mice. They noted, however, that increased gut permeability in *IL17a*$^{-/-}$ mice correlated with abnormal subcellular localization of occludin, a tight junction protein. The gut-protective role of IL-17A was abrogated in mice that lacked expression of the IL-17 receptor adaptor protein Act-1 (encoded by *Traf3ip2*) in epithelial cells, further supporting the key role of IL-17A in maintaining barrier integrity. Finally, Lee et al. showed that γδ T cells were the major source of IL-17A in the gut. Interestingly, IL-17A production by these cells was independent from IL-23 signaling implying that IL-23-blockade does not affect IL-17A production from innate cells[78]. Maxwell et al. reported similar findings using the multidrug resistance-1a-ablated (*Abcb1a*$^{-/-}$) mouse model of colitis[79]. They demonstrated that IL-17A and IL-17RA blockade exacerbated disease in this

model, while blockade of IL-12/IL-23 p40 or IL-23 p19 resulted in disease protection. Together, these data demonstrate that IL-17A is involved in tissue repair and does not drive pathogenic inflammation in the gut, in contrast to what has been shown for other chronic inflammatory diseases. These findings are relevant because sub-clinical gut inflammation is a common feature of SpA patients[80, 81]. To determine if IL-17A inhibition could result in increased numbers of IBD cases, a very recent study has evaluated the incidence rates of IBD in a total of 7355 patients treated with IL-17A inhibitors for psoriatic arthritis (PsA), Pso or SpA. In the per year analysis, the exposure adjusted incidence rates (EIARs) did not increase over time in patients treated with anti-IL-17A[82].

In contrast with the failure of IL-17 blockade, the inhibition of IL-12/23 with ustekinumab or risankizumab in moderate to severe Crohn's disease resulted in significantly higher response rates compared to placebo[83, 84], suggesting that the pathogenic activity of IL-23 in this disease cannot be explained by the simple induction of IL-17, and indicating that the pro-inflammatory cytokine activity is context-dependent.

AS shares with Crohn's disease the association with several loci linked to the IL-23/IL-17 pathway, such as *IL23R*[9, 10, 17], and IL-23 overexpression induced an AS-like phenotype in an animal model[43], suggesting an involvement of this cytokine in human AS and prompting the design of clinical studies to test the efficacy of IL-23 blockers. However, the IL-23 inhibitor risankizumab failed to demonstrate any significant clinical improvement in patients with active AS, despite the reduction of CRP, an inflammation marker[85]. These findings were unexpected in the light of the effectiveness of IL-17A inhibitors in the same disease[86, 87].

The expansion of several population of IL-17 producing immune cells have been associated with AS, including CD4+ Th17 cells[88, 89], KIR3DL2-expressing T cells that can engage cell-surface HLA-B27 homodimers[90], and innate cell populations, such as IL-17-producing γδ T cells that express the IL-23R[42]. Baeten and colleagues demonstrated that inhibiting IL-17A in 30 randomly assigned AS patients with secukinumab significantly reduced clinical and biological signs of active AS when compared to placebo and had a good safety profile[86]. These results were confirmed in two subsequent phase 3 trials[87], and this treatment is now recommended for the treatment of patients with axial spondyloarthritis that fail treatment with TNF-inhibitors[91]. Phase 3 trials have also documented efficacy of anti-IL-17A therapy for the treatment of psoriatic arthritis[92-94].

The efficacy of anti-IL-17A therapy in psoriasis and AS but its failure in Crohn's disease raised questions about the mechanism of action of this drug. We have recently started to address this issue in collaboration with the team of D. Baeten and analyzed the effects of IL-17A inhibition on the immunopathology of target lesions and systemic immune responses in peripheral SpA[95]. We observed that clinical improvement in joint counts was associated with decreased synovial expression of *IL17A* but not of *TNF* transcripts and with a histologic decrease in synovial sublining macrophages and neutrophils. Anti-IL-17A treatment

decreased the inflammatory markers CRP and ESR, as well as MMP-3 production in whole-blood stimulation assays with SEB and zymosan as stimuli. We also noted a marked reduction of IL-17A itself. However, the capacity of peripheral blood cells to produce additional cytokines and chemokines upon stimulation with SEB and zymosan did not change after anti-IL-17A therapy. We concluded that clinical improvement upon anti-IL-17A treatment was paralleled by immunomodulation of inflamed target tissues without compromising systemic immune responses[95].

Blocking IL-17 is an effective therapeutic approach in SpA, however which cells are responsible for IL-17 production in this disease is still debated. Recently, the attention has been focused on the innate arm of the immune response, in particular on the role of type 3 innate lymphoid cells (ILC3s), which have been characterized as important for the secretion of proinflammatory cytokines, such as IL-17A and IL-22, in several different tissues. In collaboration with the team of D. Baeten we have recently analyzed the synovial tissue of patients with peripheral SpA for the presence of ILC subsets, and tested the cytokine production of these cells. The analysis of matched synovial tissue (ST), synovial fluid and peripheral blood from SpA patients with actively inflamed knee joints showed that ILCs, and in particular NKp44^{+} ILC3s, are expanded in inflamed arthritic joints. Single-cell gene expression analysis demonstrated that ILCs infiltrating the synovia were clearly distinguishable from T cells in the same tissue, as well as their peripheral blood counterparts. A large fraction of ST ILC3s expressed signature transcripts of the IL-23/IL-17 pathway, including *RORC*, *AHR* and *IL23R*, and secreted IL-22 and CSF2 upon *in vitro* stimulation, however they did not produce IL-17A. This study demonstrated that ILC3s are absolutely and relatively enriched in the synovial joint of patients with SpA, but they are not a significant source of IL-17A in this tissue[96], indicating that additional studies are needed to define the cellular sources of IL-17A in this disease.

The results of clinical trials targeting TYK2 and cytokines of the IL-23/IL-17 axis have been very encouraging and have increased the treatment options for several chronic inflammatory diseases. However, the unexpected failures of anti-IL-23 in AS and of anti-IL-17A in Crohn's disease remind us of our incomplete understanding of the pathogenic mechanisms of these diseases and of the biology of the IL-23/IL-17 pathway in humans. Achieving better therapeutic outcomes for more patients will require continued investment in, and enhanced collaboration between, fundamental and translational science.

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Conflict of interest

The authors declare no conflict of interest.

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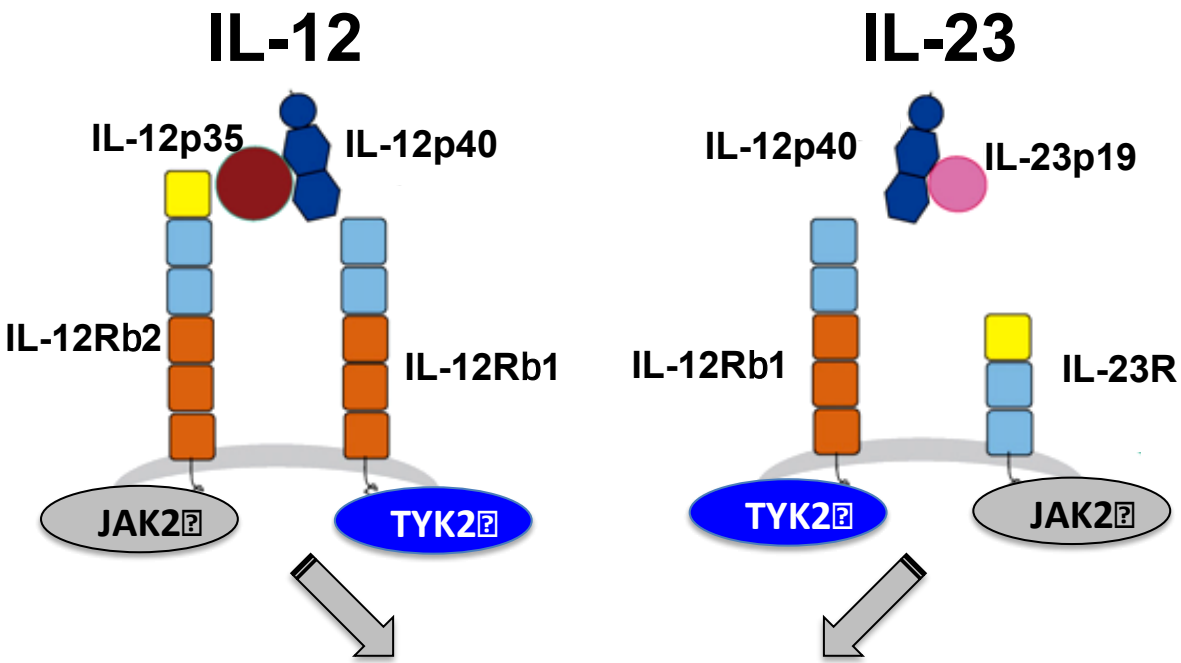
Figure legends

Figure 1: The effect of genetic variation at *TYK2* rs34536443 on signaling in response to IL-12 and IL-23 and inflammatory or infectious disease susceptibility. The upper panel shows a schematic representation of the IL-12 and IL-23 signaling pathways[26, 97]. The table summarizes data from Dendrou et al. and Boisson-Dupuis et al.[51, 55]. See text for detail.

Figure 2: Human Th17 cell differentiation and drugs targeting this pathway. Shown are several small molecule inhibitors in clinical development (in grey) targeting the RORγt transcription factor and approved (in black) monoclonal antibodies targeting IL-23 or IL-17.

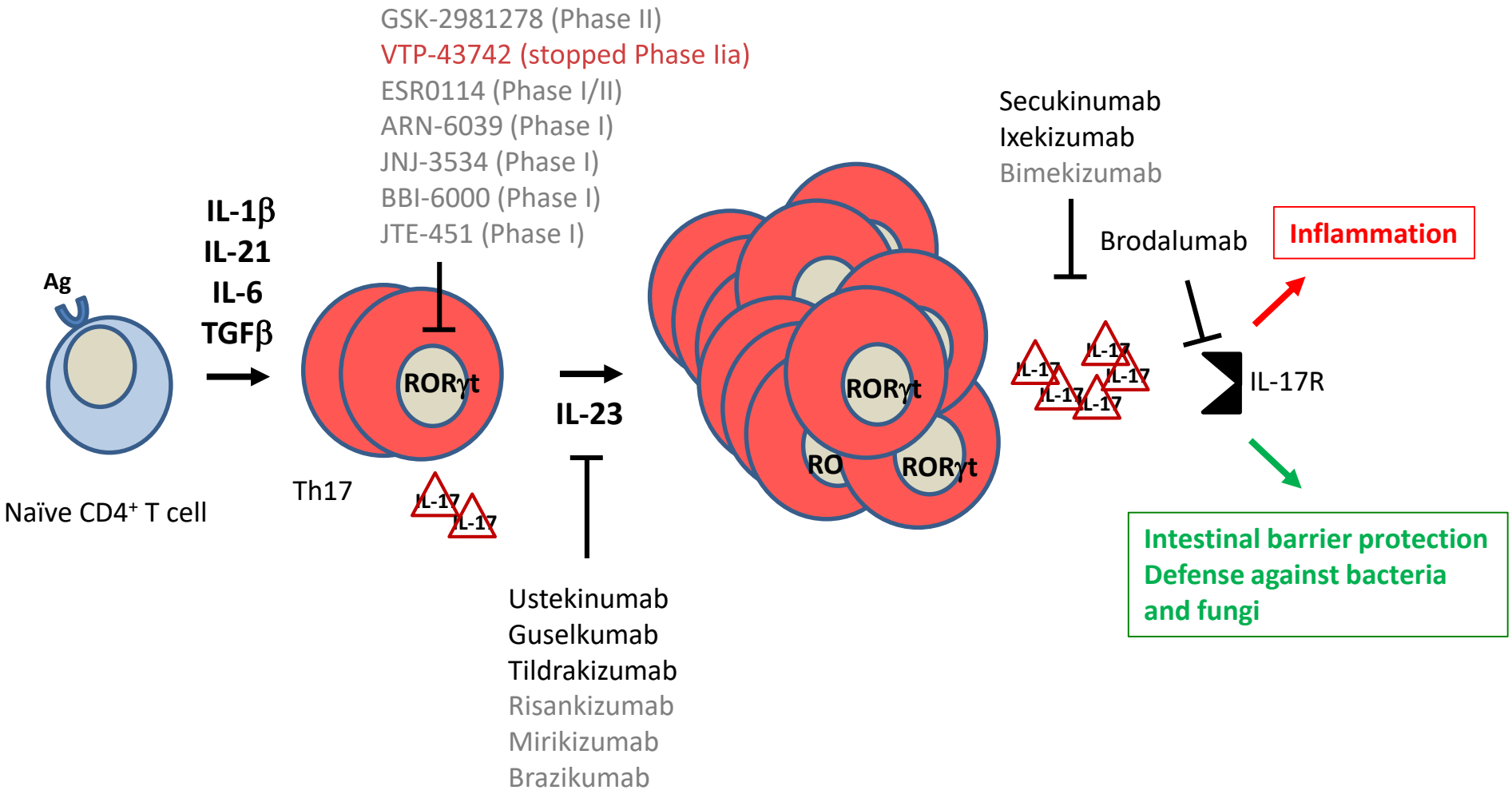
Fig. 1



TYK2 protein (genotype)	STAT4 activation	STAT3 activation	Inflammatory disease	Infections
Pro1104/Pro1104 (rs34536443: *G/G*)	+++++	+++++	At risk	Low risk
Pro1104/Ala1104 (rs34536443: *G/C*)	++++	++++	Lower risk	Low risk
Ala1104/Ala1104 (rs34536443: *C/C*)	++ (*1*), ++++ (*2*)	+ (*1*), + (*2*)	Very low risk	TB, MSMD

(1) Dendrou *et al., Sci. Transl. Med.* (2016)
(2) Boisson-Dupuis *et al., Sci. Immunol.* (2018)

Fig. 2


GSK-2981278 (Phase II)
VTP-43742 (stopped Phase Iia)
ESR0114 (Phase I/II)
ARN-6039 (Phase I)
JNJ-3534 (Phase I)
BBI-6000 (Phase I)
JTE-451 (Phase I)
Secukinumab
Ixekizumab
Bimekizumab
Brodalumab
Inflammation
Ag
IL-1β
IL-21
IL-6
TGFβ
RORγt
IL-23
IL-17
IL-17R
Naïve CD4⁺ T cell
Th17
Ustekinumab
Guselkumab
Tildrakizumab
Risankizumab
Mirikizumab
Brazikumab
Intestinal barrier protection
Defense against bacteria
and fungi