The role of innate immune cells in tissue inflammation in spondyloarthritis

Noordenbos, T.

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GENERAL INTRODUCTION

IMMUNE MECHANISMS: INNATE IMMUNITY

Troy Noordenbos and Dominique Baeten

Department of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam, the Netherlands

INTRODUCTION TO INNATE IMMUNITY

Immune-mediated inflammatory diseases (IMIDs) can be classified in different ways based on their genetic architecture [1, 2], their organ-specific or systemic nature, the involvement of specific cytokine pathways [3], or other cellular and molecular features (Fig. 1). Although each of these classifications can be useful, they are also artificial and tend to oversimplify the complexity of these disorders.

A conceptually useful classification was proposed by McGonagle and McDermott [4]: the authors identify two extremes with, on the one side, pure autoimmune disease caused by recognition of self-antigens by the adaptive immune system and, on the other side, autoinflammatory syndromes caused by a disproportional activation of mainly innate cytokine pathways. The extremes are represented by rare monogenic diseases. A common example for a pure autoimmune syndrome is autoimmune polyendocrine syndrome-1 (APS-1), which is caused by a loss-of-function mutation of the molecule AIRE, resulting in abnormal selection of autoreactive T and B lymphocytes. A common example of a pure autoinflammatory syndrome is TNF-receptor-associated periodic syndrome (TRAPS), caused by a mutation in TNF-receptor I.

Figure 1. Clustering of immune-mediated diseases based on acquired versus innate immune mechanisms. Rare monogenic diseases define the extremes of a spectrum between disease phenotypes that are caused by, on the one hand, problems in specific recognition of self-antigens by the adaptive immune system and, on the other hand, uncontrolled or inappropriate activation of innate cytokine pathways. A selection of common diseases is placed in the spectrum, based upon gender bias, genetic associations, and response to treatments. FMF, Familial Mediterranean fever; TRAPS, TNF receptor-associated periodic syndrome; HIDS, hyperimmunoglobinaemia D with periodic fever syndrome; PAPA, pyogenic arthritis pyoderma gangrenosum and severe cystic acne; ALPS, autoimmune lymphoproliferative syndrome; IPEX, immune dysregulation polyendocrinopathy enteropathy X-linked; APECED, autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy syndrome; Sarco, sarcoidosis; CU, colitis ulcerosa; Pso, psoriasis; T1D, type 1 diabetes; RA, rheumatoid arthritis; PBC, primary biliary cirrhosis; SLE, systemic lupus erythematosus.
In the continuum between these monogenic autoimmune versus autoinflammatory disorders, common polygenic IMIDs cluster roughly into two groups, based on predominant disease mechanisms. The cluster of autoimmune diseases includes rheumatic diseases such as systemic lupus erythematosus and RA. These diseases share prototypical autoimmune features such as female predominance, autoantibodies, genetic polymorphisms in B- or T-lymphocyte-related molecules (MHC class II molecules, co- stimulation, B cell receptor (BCR) and TCR, PTPN22), and clinical response to lymphocyte- targeting therapies such as blocking of T- cell co-stimulation (CTLA-4-Ig) and B- cell depletion (anti- CD20). On the other side of the spectrum is a cluster of diseases that can be described as mainly autoinflammatory. Examples are gout, psoriasis, and IBD. These diseases display no female predominance, lack disease- specific autoantibodies, are genetically associated with proinflammatory cytokine pathways (TNF, IL-1, and IL-23), and respond clinically to blocking these cytokine pathways. Over the last decade, several lines of evidence suggest that SpA belongs to the cluster of autoinflammatory diseases despite its strong genetic linkage to the MHC class I molecule HLA- B27. This association with HLA- B27, as well as more recently discovered genetic associations with genes encoding various aminopeptidases responsible for truncating peptides in preparation for MHC class I presentation, fueled the hypothesis that HLA- B27- restricted presentation of antigens (possibly autoantigens) to CD8+ cytotoxic lymphocytes is central to the pathogenesis of the disease. This hypothesis, however, has never been formally demonstrated. On the contrary, two studies showing that CD8+ cells are not required for disease development in the HLA- B27 tg rat model of SpA challenged this ‘adaptive’ theory [5, 6]. This has initiated a large amount of research in the role of other cell types in general and in innate immune responses in particular in the pathophysiology of SpA. This chapter reviews the evidence supporting the role of innate immune alterations and autoinflammatory mechanisms in the pathogenesis of SpA. It consecutively discusses (1) the potential role of HLA- B27 in the innate immune response, (2) genetic associations with innate cytokine pathways and the role of these innate cytokine pathways in animal models of SpA, and (3) the presence and function of innate immune cells in target tissues of human SpA.

HLA- B27

Although environmental triggers might be crucial in the induction of disease, genetic risk factors are estimated to account for 80–90% of the susceptibility to AS [7]. HLA- B27 confers at least 25% of this risk, indicating the central role of this molecule in the pathophysiology of SpA. Several hypotheses have been proposed to explain the association of HLA- B27 with the disease (Fig. 2).

ARTHROGENIC PEPTIDE HYPOTHESIS

HLA- B27 can, like all other MHC class I molecules, present a restricted set of peptides to cytotoxic T cells [8]. The arthritogenic peptide theory proposes that HLA- B27 binds a unique peptide derived from a microorganism and elicits a cytotoxic T- cell response cross- reactive
Figure 2. Three theories on the contribution of HLA-B27 to SpA pathophysiology. HLA-B27 forms a heterotrimeric complex with peptide and β2m, known as the presentation complex. The arthritogenic peptide hypothesis proposes that HLA-B27 binds a restricted peptide that is recognized by autoreactive CD8 T cells. The second theory states that misfolding of HLA-B27 occurs during formation of the presentation complex. A stress reaction is induced that is known as the unfolded protein response. This response includes upregulation of the transcription factor CHOP, which leads to expression of IL-23A. The third theory describes that the presentation complex can be internalized and that free forms of HLA-B27, including a homodimer, can be re-expressed on the cell surface. KIRs expressed on CD4+ T cells or NK cells can be triggered upon recognition of free heavy chain forms of HLA-B27. The recently discovered interaction of HLA-B27 with ERAP1, can be important for the creation of a restricted specific peptide described in the arthritogenic peptide hypothesis. Alternatively, ERAP1 is known to influence peptide availability, thus influencing the folding kinetics in the ER, which can lead to misfolding. ERAP1, Endoplasmic reticulum associated aminopeptidase 1; CHOP, C/EBP homologous protein; IL-23, interleukin 23; KIR3DL2, killer immunoglobulin receptor 3DL2.

with a self-peptide in the context of HLA-B27 [9]. Although HLA-B27-restricted cytotoxic T cells reactive to enteric bacteria were found in the synovial fluid of ReA patients [10, 11], the autoimmune nature of these cells has not been convincingly demonstrated. A strong argument against the arthritogenic peptide hypothesis comes from a rat model where overexpression of HLA-B27 induces spontaneous SpA-like disease [12]. Genetic deletion as well as depletion of CD8+ cytotoxic T cells did not prevent or modify disease compared to wild type [5, 6]. Additionally, a specific arthritogenic peptide has never been identified in human studies. Recently, SNPs in the genes encoding three aminopeptidases, ERAP1, ERAP2, and aminopeptidase puromycin sensitive NPEPPS, were found to be associated with AS susceptibility [13]. This finding seems to favour a role for altered antigen presentation by HLA-B27 because these molecules are important for trimming peptides for optimal loading onto MHC molecules. Alternatively, it should be considered that these molecules have a strong influence on peptide availability and thus on the kinetics of the formation of the heterotrimeric complex HLA-B27/β2m peptide [14–16], favouring an alternative hypothesis (see sections ‘HLA-B27 misfolding and endoplasmatic reticulum stress’ and ‘HLA-B27 homodimers and KIR triggering’). Interestingly, the association of ERAP1 is
restricted to HLA-B27-positive AS, whereas ERAP2 is not [17]. A similar interaction is seen for ERAP1 and MHC class I molecules in psoriasis (HLA-C) [18] and Behçet’s disease (HLA-B*51) [19], suggesting that a similar mechanism is underlying the HLA association in the different diseases.

**HLA-B27 MISFOLDING AND ENDOPLASMATIC RETICULUM STRESS**

HLA-B27 has a low folding speed compared to related but not SpA-associated MHC class I molecules, which leads more frequently to misfolding [20]. To avoid accumulation of misfolded proteins in the ER, continuous degradation by proteasome-dependent mechanisms takes place. When normal compensatory mechanisms cannot keep up with the load of misfolded protein, a stress response is elicited in the ER, known as the unfolded protein response. An interesting link between ER stress and SpA is that the ER-stress-induced transcription factor C/EBP homologous protein (CHOP) can directly enhance IL-23p19 expression in, for example, dendritic cells [21]. As discussed in the section ‘IL-23/IL-17’ in detail, there is now clear pathophysiological and clinical evidence for a key role of the IL-23/IL-17 pathway in SpA.

A causative relation between HLA-B27 expression and the unfolded protein response was detected in vivo in the HLA-B27 tg rat model. In bone marrow-derived macrophages from these animals, HLA-B27 misfolding was detected [22] and this correlated with production of IL-23 [23]. In SpA patients, however, the relation between HLA-B27 and the unfolded protein response is not easily detected [24–26], possibly because this process is occurring in specific cell types and in specific disease localizations. Another proposed mode of action is that HLA-B27 misfolding activates autophagy pathways [27]. Interestingly, SpA is clinically and pathophysiologically related to CD, which is convincingly associated with autophagy-related genes, the strongest association of which is ATG16L1 [28]. Although SNPs in ATG16L1 are not associated with AS [29], two other genes that are associated with SpA, NPEPPS and GPR37, do play a role in autophagy [13, 30]. Overexpression of both molecules induces autophagy in model systems [31, 32], but the exact role of autophagy in SpA remains to be investigated in detail.

**HLA-B27 HOMODIMERS AND KIR TRIGGERING**

Another molecular feature of HLA-B27 is that, when not properly complexed with β2m, it can form homodimers due to its unpaired cysteine residue and this can be expressed in this conformation on the cell membrane [33, 34]. Additionally, the heavy chain can be expressed on the plasma membrane as a monomer. These alternative forms of HLA-B27 heavy chains can be a ligand for innate immune receptors on myeloid cells, T cells, and NK cells. These receptors include KIR3DL1, KIR3DL2, and LILIRB2 in humans [35] and in rodent PIR [36]. The interaction between KIR3DL2 and HLA-B27 has been shown to play a role in SpA as it results in direct immune activation of T cells, especially T_{H}17 cells, and NK cells [37–39]. Genetic associations with SpA were not detected for KIR3DL2, although they have
been suggested for KIR3DL1 and KIR3DS1 in lower-powered studies in Asia [40]. As for the unfolded protein response hypothesis, this homodimer theory proposes an antigen-independent, innate role for HLA-B27.

**HLA- B27 AND THE MICROBIOME**

A link between GI bacteria and SpA was established several years ago. The HLA- B27-associated ReA subtype of SpA is triggered by GI infections with *Shigella, Salmonella, Yersinia*, and *Campylobacter or urogenital infections with Chlamydia* [41– 43]. Also, HLA- B27 tg rats fail to develop disease when kept in germ-free conditions [44]. The underlying mechanisms are still unclear, but a direct influence of HLA- B27 on the microbiome has been recently proposed based on the concept that alterations in the microbiome can steer inflammatory diseases, possibly through promotion of T\(_{\text{H}}\)17 responses [45, 46]. In the HLA- B27 tg rat model, mono-association with *Bacteroides vulgatus*, but not with certain other strains, was sufficient to rescue the colitis phenotype in these animals. Unrestricted bacterial flora profiles were significantly different between non-tg and HLA- B27/β2m tg rats [47]. Taken together, this suggests that HLA-B27 can restrict or alter the gut microbiome.

In patients, it has been challenging to characterize the microbiome, but the first data from 16S rRNA sequencing showed dysbiosis in AS patients versus controls [48]. A causative relation between altered gut microbiome and disease is still speculative and microbiota could very well be secondary to the disease process [49]. Moreover, bacterial triggers in the gut could also act merely as non-specific adjuvant-like danger signals, rather than modulate the immune response in a specific way. Supporting this concept, we recently demonstrated that immunization with low amounts of TLR-ligands such as heat-killed *Mycobacterium tuberculosis* is sufficient to trigger spondylitis and arthritis, with a more than 80% incidence in HLA-B27/β2m tg rats but not in control animals. In vitro studies using splenocytes from these rats demonstrated reactivity to innate immune stimulation by increased production of IL-1α and β [50]. Independently of the exact mechanism, these data collectively indicate that bacterial stress and innate immune activation play important roles in triggering HLA-B27-associated disease.

**GENETIC ASSOCIATIONS WITH INNATE CYTOKINE PATHWAYS**

Genetic association with cytokines, receptors, and intracellular signalling molecules have identified IL-1, TNF, and IL-23/IL-17 as major cytokine pathways for the pathophysiology of SpA. The innate nature of these pathways is stressed by monogenic human diseases affecting these cytokine pathways in the context of a stable adaptive immune system.

**IL-1**

Innate triggering through pattern recognition receptors on mainly macrophages and dendritic cells stimulates the production of pro-IL-1β, which is consequently cleaved to
IL-1β by the inflammasome and released to cause a proinflammatory environment. GWAS in AS indicated an association with the IL-1R1-IL-1R2 locus (13, 51). IL-1R1, encoding for the signalling receptor, and IL-1R2, encoding for a decoy receptor, can directly modulate the proinflammatory potential of IL-1α and IL-1β. In parallel, family-based association testing found the IL-1α locus to be associated with SpA [52]. Two open-label trials blocking IL-1 signalling in SpA did not show a clinically relevant effect [53, 54], though a definite conclusion can only come from larger study populations, randomized controlled methodology, and maybe a new generation of IL-1 blockers. In mice, however, deletion of IL-1 receptor-antagonist (IL-1Ra−/−), resulting in unopposed and thus amplified IL-1 signalling, induced erosive arthritis, aortitis, T-cell-independent psoriasis-like disease, and intervertebral disc degeneration [55–57]. Interestingly, genetic deletion or neutralization of IL-17A in these mice prevented disease [58, 59] and, when crossed on an RAG2−/− background, these mice developed severe IL-17-mediated colitis [60]. This suggests that innate induced IL-1 can fuel the IL-23/IL-17 pathway, which is discussed in the section ‘IL-23/IL-17’. In a different arthritis model, the human TNF-tg mice, IL-1α and/or IL-1β mediate TNF-induced bone and cartilage destruction [61]. Taken together, IL-1 is genetically linked to SpA and molecularly linked to arthritis, probably in a T-cell-independent manner.

**TNF**

Three genetic associations with AS were found in the TNF-pathway, TNFRSF1A [62–65], TRADD [66], and TNFSF15 [67]. TNFRSF1A encodes TNFR1, an important transmembrane receptor involved in TNF signalling. TRADD is an adaptor protein that associates with the cytoplasmic domain of TNFR1, overexpression of which leads to apoptosis and activation of NFκB [68]. The cytokine TNFSF15 can be induced by IL-1 and TNFα and can associate with Death receptor 3 to promote Th17-mediated disease [69, 70]. Clinical inhibition of TNF is efficacious in SpA, providing the strongest evidence of the importance of the TNF pathway [71, 72].

Despite this overwhelming evidence that TNF is a central driver of the inflammatory process in SpA, it remains still largely unknown which cells produce TNF in SpA and which cells will respond to this altered TNF production. Macrophages and other myeloid cells are generally considered the major producers of TNF, but this remains to be confirmed in the context of SpA. As to the effects of TNF on other cell types, it is clear that TNF can directly and/or indirectly activate osteoclasts as TNF blockade halts joint destruction in SpA [73]. However, TNF blockade fails to halt new bone formation in AS [74, 75] and the relation between TNF and osteoproliferation remains uncertain.

In this context, various animal models of TNF overexpression have provided important insights in the potential role of TNF in SpA. Mice overexpressing human TNF develop a severe systemic inflammation and destructive polyarthritis reminiscent of human RA, but do not develop SpA features [76]. This destructive phenotype could be reversed by blocking DKK-1, a negative regulator of the Wnt pathway [77, 78], thereby indicating that TNF not only activates bone destruction but also impedes new bone formation through inhibitors of the Wnt pathway. The exact relevance for human SpA remains unclear, since not only TNF but
also other cytokines such as IL-6 regulate DKK-1 [79]. Similar to mice overexpressing human TNF, the TNFΔ ARE mice, in which murine TNF is overexpressed by modulation of mRNA stability, also demonstrate a destructive polyarthritis reminiscent of RA [80]. This model, however, also develops colitis and both manifestations are dependent on TNFR1 [81]. More recently, this model was also shown to develop some enthesitis and to be dependent on mechanical stress [82]. Although the model fails to faithfully recapitulate key SpA features such as spondylitis and new bone formation, it does contribute to deciphering the role of TNF in the pathogenesis of SpA.

The third and most recent model consists of mice that overexpress selectively the transmembrane but not the soluble form of TNF [83]. TNF is formed as a 26-kDa transmembrane molecule which is then cleaved by ADAM metallopeptidase domain 17 (ADAM17) from the membrane and released in a 17-kDa soluble form. Both soluble TNF (sTNF) and transmembrane TNF (tmTNF) can bind TNF receptors and are biologically active. Interestingly, mice overexpressing selectively tmTNF developed a completely different phenotype than the two other models: they did not show signs of systemic inflammation or destructive polyarthritis but developed spondylitis and deforming arthritis. At histology, the animals demonstrated synovitis as well as enthesitis and osteitis, which was accompanied by some bone destruction and, most interestingly, also by pronounced axial and peripheral new bone formation. Radiology confirmed progression towards total joint ankylosis. This model thus seems to phenocopy the musculoskeletal manifestations of SpA and for the first time provides a link between TNF in its transmembrane form and new bone formation. It is likely that this mechanism is also relevant for human SpA, as clearly the balance is in favour of tmTNF versus sTNF in SpA versus RA synovitis [84].

**IL-23/IL-17**

SNPs in IL-23R are strongly associated with AS, psoriasis, and IBD [51, 85], suggestive of a common mechanism across these diseases. In AS, the recent discovery of additional associated genes for signalling molecules directly downstream of the IL-23 receptor, TYK2, JAK2, and STAT3, provides further evidence for an involvement of the IL-23/IL-17 pathway in SpA (Fig. 3) [13]. We previously indicated in the section ‘HLA-B27’ that research on the functional role of HLA-B27 has linked both HLA-B27 misfolding in the ER and HLA-B27 homodimer expression on the cell membrane to activation of the IL-23/IL-17 axis. Direct evidence for the involvement of these cytokines in the pathogenesis of SpA has now been provided by the positive results of clinical trials with targeted therapies directed towards either IL-23 or IL-17 in AS and PsA [86–90]. The IL-23/IL-17 pathway has been studied in detail in animal models, where IL-23 production by myeloid cells (mainly dendritic cells) steers an IL-17 response by specialized T<sub>h</sub>17 cells. It needs to be emphasized, however, that even in animal models many different immune cells can respond to IL-23 by IL-17 production [91].

In the Introduction we discussed evidence that T cells may not be that crucial in the pathogenesis of SpA, and this raises the question of which cell types are involved in the IL-23/IL-17 axis in SpA. IL-23 consists of two subunits, p40 and p19. The p40 subunit is
ectopic overexpression (microcircle DNA) in B10.RIII mouse

Innate trigger:
> Spontaneous disease, but not under SPF conditions
> Induction by Curdlan, a ligand for Dectin-1

SKG-mouse
Innate trigger:
> Spontaneous disease, but not under SPF conditions
> Induction by m. Tuberculosis

HLA-B27tg rat
Innate trigger:
> Spontaneous disease, but not under SPF conditions

Overexpression of IL-23:
Synovitis, bone loss, enthesitis, aortitis. Both αIL-17 and αIL-22 reduces symptoms

Overexpression of IL-17:
No phenotype!

Overexpression of IL-22:
Synovitis, activation of osteoblasts

General disease symptoms:
Synovitis, enthesitis, spondylitis, ileitis

αIL-23 severely reduces all symptoms
αIL-17− diminished symptoms
αIL-22 diminished symptoms, but aggravate ileitis

General disease symptoms:
Synovitis, enthesitis, spondylitis, osteitis, new bone formation, osteoporosis

αIL-17 diminished symptoms

αIL-23 p40 is clinically effective
αIL-17 is clinically effective
αIL-17R is clinically effective

Overexpression of IL-22:
Synovitis, bone loss, enthesitis, aortitis.
Both αIL-17 and αIL-22 reduces symptoms

Human SpA
Innate trigger:
> Reactive arthritis
> Endogenous triggers
> Gut microbiome?

General disease symptoms:
Synovitis, enthesitis, spondylitis, ileitis

αIL-23 severely reduces all symptoms
αIL-17− diminished symptoms
αIL-22 diminished symptoms, but aggravate ileitis

General disease symptoms:
Synovitis, enthesitis, spondylitis, osteitis, new bone formation, osteoporosis

αIL-17 diminished symptoms

αIL-23 p40 is clinically effective
αIL-17 is clinically effective
αIL-17R is clinically effective

Figure 3. IL-23/IL-17/IL-22 axis in experimental and human SpA. This figure summarizes the relative contribution of IL-23, IL-17, and IL-22 in various models of experimental and human SpA. The graphic models two theories on human SpA. First, IL-23 is produced ectopically, for example in the gut by monocytic phagocytes upon a bacterial trigger from the gut microbiome (concordant with the IL-23 overexpression model), or second, IL-23 is produced locally upon endogenous triggering of innate receptors and/or cellular stress (concordant with absence of systemic elevation of IL-23 in human SpA). IL-23 leads to activation of restricted responsive cells to produce locally IL-17 and/or IL-22. IL-17 can than drive inflammation and bone erosion, and IL-22 can drive bone anabolism. JAK, Janus kinase; STAT3, signal transducer and activator of transcription 3; ROR-γ, RAR-related orphan receptor gamma; ILC3, innate lymphoid cell group 3; SKG-mouse, mutation in skg locus, fine-mapped to ZAP70; HLA-B27tg rat, overexpression of human HLA-B27 and β2m; αIL-23/αIL-23p40/αIL-17/αIL-17R/αIL-22, treatment with neutralizing antibodies; IL-17−, animal with a deletion of the IL-17a locus.

shared with IL-12 and p19 seems to be specific for IL-23 [92]. Murine studies have revealed that deletion of either the p40 or the p19 subunit, but not the unique subunit of IL-12 (p35), protected mice from various autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE) [93, 94] and collagen-induced arthritis (CIA) [95, 96]. Elegant mouse studies have identified monocytic phagocytes in the gut, such as CD11b+ dendritic cells and
macrophages, as the natural cellular source of IL-23 during early stages of infection with *Citrobacter rodentium* [97, 98]. Accordingly, it was proposed that gut-derived IL-23 may lead to increased blood levels and then act locally in target tissues on IL-23R-positive cells [99]. In human SpA, however, systemic IL-23 levels are not elevated and do not correlate with disease activity [100, 101]. Moreover, peripheral blood-derived macrophages and dendritic cells derived from SpA patients do not produce higher levels of IL-23 upon TLR stimulation than seen in healthy controls [102, 103].

Independently of the exact origin of IL-23, there is now clear experimental evidence that it plays a direct role in the pathogenesis of the disease. Systemic overexpression of IL-23 by microcircle technology induced a severe destructive polyarthritis [104] as well as enthesitis [105] in mice. In the first model, the disease appeared to be myeloid-cell dependent. In the second model, the IL-23-responsive cells appear to be entheses-resident, CD4–CD8 double-negative ‘innate-like’ lymphocytes. Both models thus point towards a role for innate rather than adaptive immune cells. Interestingly, the expression of the IL-23 receptor in these innate cell populations in tissues is constitutive, in contrast to the T-helper cells where the expression is restricted [106]. It is hypothesized that these innate IL-23 receptor-expressing cells are important for a rapid response against various pathogens and also for gut homeostasis via the production of IL-22 [97, 98, 107].

Another animal model that has helped to better understand the role of IL-23/IL-17 in SpA is the curdlan-induced disease in SKG mice. SKG mice have a mutation in the TCR zeta chain and develop autoimmune phenomena [108]. When immunized with high doses of curdlan, a TLR agonist, this mouse strain develops arthritis, enthesitis, spondylitis, and ileitis, confirming the central role of innate immune triggering which is also observed in the HLA-B27 rat model. Interestingly, preventive and therapeutic blockade of IL-23 suppressed all disease features in this model. Genetic deletion of IL-17A also had a significant, albeit limited, beneficial effect on all disease symptoms. Inhibition of another IL-23-driven cytokine, IL-22, however, only ameliorated enthesitis, had no effect on the arthritis and spondylitis, and even worsened the ileitis [109]. IL-17 inhibition was also recently tested in the HLA-B27 tg rat model [110]; preliminary data indicate that a preventive treatment with a IL-17A blocking antibody not only reduced clinical and histological signs of axial and peripheral inflammation but also suppressed radiographic and histological new bone formation.

Collectively, the data from genetics, experimental models, and clinical studies strongly indicate a key role for the IL-23/IL-17 pathway in SpA. In contrast to other diseases, however, it seems that the role of innate immune cells is more pronounced than that of canonical T₁₇ cells in this disease. This concept is further supported by analysis of human blood and tissue samples, as described in the section ‘Innate immune cells in target tissue’.

**INNATE IMMUNE CELLS IN TARGET TISSUES**

**Axial tissue**

Analysis of target tissue in axial SpA has been challenging, because tissue sampling is relatively invasive and is usually restricted to surgery for a clinical indication. However,
tissues from intervertebral discs, SI joints, zygapophyseal joints, and the manubriosternal junction have been studied. Initial histopathological analysis of SI biopsies from patients with active disease revealed marked signals for T cells and macrophages [111, 112] and a strong signature for TNFα mRNA. In a controlled study, this T-cell infiltration was shown to be specific for AS and was present after longer disease duration in zygapophyseal joints obtained during kyphosis correction surgery [113]. Later, more targeted approaches identified specific molecules in the IL-23/IL-17 axis in these tissues (Table 1). IL-23 could be detected in the subchondral bone marrow and in the fibrous tissue replacing the bone marrow of AS patients. The expression was found in macrophages and MPO+ cells [114]. Immunostaining for IL-17A in these tissues revealed, surprisingly, that the cytokine is mainly found in MPO+ cells, CD15+ neutrophils, and to a lesser extent mast cells, as opposed to very rare in T cells. With the strong linkage to the IL-23/IL-17 axis in mind, these data from axial tissues argue for a role for innate immunity, rather than for T cells.

Peripheral tissue
Researchers generally have an easier access to tissues of peripheral joints and extra-articular structures of SpA patients and controls. Inflammatory conditions of the synovial membrane result in massive infiltration of immune cells, neovascularization, and proliferation of the intimal lining. The extent of changes related to generalized inflammation often precludes identification of disease-specific mechanisms. Using other forms of arthritis as control for SpA, like RA, gout, or osteoarthritis, effects of general inflammation can be filtered out and disease-specific cellular and molecular signatures can be identified. Surprisingly, the number of immune cells expressing cell-specific antigens like CD68 (macrophages), CD3 (T cells), and CD20 (B cells) were similar between diseases [115, 116]. However, the number of CD163-expressing macrophages and C-kit/tryptase-positive mast cells was specifically increased in SpA [116, 117]. CD163 is a scavenger receptor for haemoglobin/haptoglobin complexes, binding and internalizing potentially toxic-free haem [118]. CD163+ macrophages in the synovium correlated with disease activity [119] and were enriched in very early stages of disease, stressing a possible role in pathogenesis. Macrophages are very plastic and they can exert different roles, ranging from a proinflammatory phenotype (M1) to an anti-inflammatory phenotype (M2). The functional phenotype upon IL-10 polarization is considered anti-inflammatory [120]. CD163+ macrophages are considered anti-inflammatory (M2), since IL-10 induces expression of CD163 in vitro [121].

This suggests that in situ CD163 macrophages induce a shift in balance towards the M2 phenotype in SpA and that these cells might be acting to counterbalance general inflammation, rather than drive inflammation. The function of this shifted balance was reflected by lower local levels of proinflammatory macrophage (M1) cytokines in the synovial fluid [122]. Although clear differences in the M1–M2 balance were found between SpA and RA, conclusions should take into account that in vitro-defined classifications, like M1 and M2, do not fully apply to in situ synovial tissue macrophages [123].

The exact role of these CD163+ macrophages in SpA has not been deciphered yet; moreover, the increased frequency of these cells could still be a bystander effect. Mast cells
are tissue-resident sentinel cells crucial for defence against various pathogens [124], and they are capable of modulating an immune response in the absence of pathogens [125]. Due to their capacity to pre-store potent mediators, like histamine, TNF, and IL-17A, these cells have an ultra-short reaction time. Interestingly, the specific infiltration of mast cells was reproduced in a cohort of patients with short disease duration and it was not reversed by effective treatment with TNFi, suggesting that these cell types function upstream of TNF and general inflammation [116].

The IL-23/IL-17 pathway has also been analysed by immunohistochemistry in peripheral tissues. Like axial tissue, T cells expressing IL-17A were not frequently found and, as in axial tissues, both mast cells and neutrophils were identified to express IL-17A [116, 126].

Table 1. Innate cells related to the IL-23/IL-17 axis in SpA.

<table>
<thead>
<tr>
<th>Protein expression of IL-17A, IL-22, IL-23R, and ROR-γ</th>
<th>Contribution to pathology</th>
<th>Controversy</th>
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<tr>
<td>γδ-T cell (136) (FACS and ELISA)</td>
<td>Peripheral blood: Present: IL-17A, IL-23R. Nr: IL-22, ROR-γ</td>
<td>In IL-23 minicircle overexpression in mice, enthesitis is driven by primary γδ-T cell (137)</td>
</tr>
<tr>
<td>ILC3 (138) (FACS)</td>
<td>Peripheral blood: Present: IL-17A, IL-22, IL-23R. Absent: ROR-γ Bone marrow: Present: IL-22, IL-23R, IL-17A Absent: IL-17A, ROR-γ</td>
<td>Nr</td>
</tr>
<tr>
<td>MPO+ cell (114) (IHC)</td>
<td>Synovial tissue (axial): Present: IL-17A Nr: IL-22, IL-23R, ROR-γ</td>
<td>Nr</td>
</tr>
<tr>
<td>Neutrophil 114 (IHC), (116) (IHC)</td>
<td>Synovial tissue (axial and peripheral): Present: IL-17A Nr: IL-22, IL-23R, ROR-γ</td>
<td>Mast cell blockade (nilotinib) is effective in peripheral SpA (140)</td>
</tr>
<tr>
<td>Mast cell (114) (IHC), (116) (IHC), (133) (Western blot, ex vivo qPCR)</td>
<td>Synovial tissue (axial and peripheral): Present: IL-17A Absent: ROR-γ Nr: IL-22, IL-23R</td>
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</tr>
<tr>
<td>Macrophage</td>
<td>Nr</td>
<td>Nr</td>
</tr>
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</table>

Nr, Not reported; ILC3, innate lymphoid cell group 3; MPO, myeloperoxidase; FACS, fluorescence activated cell sorting; ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry.
However, the ability of both cells types to actually produce IL-17A is debated and there are conflicting data, not only in human tissue but also in more mechanistic studies in mice. In the initial reports of the knock-in reporter mice for IL-17a, IL-17f, IL-23R, or RORc, mast cells and neutrophils were not identified among cells expressing the promoter of interest [91, 127–129]. However, a recent publication detected ROR-γt and IL-17A expression in bone marrow neutrophils from reporter knock-in mice [130]. Moreover, this report extended these mechanistic principles to human neutrophils isolated from peripheral blood, showing that stimulation with high levels of IL-6 in combination with IL-23 induces IL-17A production dependent on RORC.

Another recent publication provides the opposite conclusion with human neutrophils isolated from peripheral blood, since investigators were not able to detect either IL-17A mRNA transcripts or IL-17A protein [131]. The ability of mast cells to produce IL-17A is equally controversial. Human cord blood-derived mast cells were able to produce IL-17A in a RORC-dependent manner [132]. However, our studies did not detect IL-17A mRNA transcripts in isolated mast cells from synovial tissue of SpA patients and from healthy donor tonsils. Instead, the mast cells were able to engulf exogenous IL-17A and store the cytokine for later release [133]. Better understanding of the role of IL-17+ neutrophils and mast cells will be crucial for the interpretation of these data in SpA. Potential other cell types have been identified in mouse studies and are proposed to be producers of IL-17A in human SpA, such as innate lymphoid cells and γδ-T cells. IL-17A-producing innate lymphoid cells are expanded in the gut, the blood, and the synovial fluid of SpA patients compared to healthy controls [134]. Moreover, cells with the phenotypic characteristics of IL-17-producing cells were found in the synovial tissue [135]. γδ-T cells are also enriched in the blood of SpA patients compared to patients with RA and healthy controls, and were shown to produce IL-17A ex vivo [136]. γδ-T cells are rarely found in inflamed synovial tissue, and it is not known if they—like in the mouse—are able to drive pathology.
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