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Pathogenesis of spondyloarthritis: autoimmune or autoinflammatory?

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ABSTRACT

Purpose of review
Spondyloarthritis (SpA) is a chronic immune-mediated inflammatory disease of unknown origin. Here we aim to review whether SpA is driven by T and/or B cell autoreactivity or by abnormal innate immune responses.

Recent findings
SpA does not share genetic risk factors, female predominance, presence of disease-specific autoantibodies and response to T or B cell targeted therapies with prototypical autoimmune diseases like rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Growing evidence indicates that increased responsiveness of innate immune cells such as macrophages, mast cells and neutrophils drives inflammation in SpA. The altered innate immune response may be related to non-antigen presenting functions of HLA-B27, including the induction of an unfolded protein response, and can be triggered by bacterial and mechanical stress. Innate immune cells appear to be the main producers of both pro-inflammatory (TNF, IL-1, IL-23, IL-17) and anti-inflammatory (IL-10) cytokines in SpA.

Summary
The predominance of myeloid above lymphoid alterations suggests an autoinflammatory rather than autoimmune origin of inflammation in SpA. Therefore, targeting innate cells or their inflammatory mediators may be more effective than T or B cell directed therapies.

Keywords
autoimmunity, autoinflammation, HLA-B27, IL-17, IL-23, spondyloarthritis
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INTRODUCTION

Why is spondyloarthritis not a typical autoimmune disease?

Autoimmune diseases are characterized by unchecked T-cell and/or B-cell reactivity to self-antigens, leading to chronic tissue inflammation. Although the causative autoantigens often remain partially elusive, prototypical autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) share a number of common features. Firstly, they display common genetic risk factors such as SNPs in molecules related to TCR/BCR signaling and central tolerance, with as prime example PTPN22 [1]. Secondly, they are characterized by a marked female predominance. Thirdly, they are associated with disease-specific autoantibodies such as anti-citrullinated protein antibodies in RA and anti-dsDNA antibodies in SLE. Finally, they respond well to T or B cell targeted treatments, such as abatacept and rituximab in RA.

An important number of immune-mediated inflammatory diseases (IMIDs) do not share these common autoimmune features, suggesting that chronic inflammation in these disorders is not primarily driven by autoreactive T or B lymphocytes. In an important conceptual manuscript, McGonagle et al proposed to classify IMIDs in a continuum ranging from autoimmunity to autoinflammation, the latter being driven by innate immune responses to specific tissue triggers, such as microorganisms or microtrauma [2] (Table 1). The best known examples of autoinflammation are the rare fever syndromes: tumor necrosis factor receptor-associated periodic syndrome (TRAPS) and familial Mediterranean fever (FMF). These diseases are caused by single gene defects affecting inflammatory cytokine pathways and leading to aberrant production of IL-1β or TNF [3]. Besides the prototypical monogenic autoinflammatory conditions, polygenic autoinflammatory diseases are viewed as the result of a combination between genetic risk factors and exogenous stress. Accordingly, these diseases respond better to cytokine blockade than to therapies targeting the acquired immune system.

Spondyloarthritis (SpA) is a polygenic IMID characterized by inflammation of the spine and peripheral joints, as well as extra-articular manifestations, such as inflammatory bowel disease (IBD), uveitis and psoriasis [*4]. SpA is not associated with typical autoimmune genes, but displays polymorphisms in genes involved in innate immune recognition (CARD9) and cytokine signaling pathways, such as the TNF (TNFRSF1A, TRADD, TNFSF15), IL-1 (IL1A, IL1R2), and IL-23/IL-17 (IL-23R, STAT3) axis [*5-8]. Moreover, SpA does not show a female predominance and is not associated with disease-specific autoantibodies. Finally, T or B cell targeted therapies such as abatacept, alefacept, efalizumab and rituximab showed very modest therapeutic efficacy in SpA [9-17] (Table 2). The lack of classical autoimmune features leads to the hypothesis that adaptive immune responses are not of primary
importance in SpA. Furthermore, the genetic background and the importance of bacterial [18] and mechanical [19] stress point towards an autoinflammatory origin of the disease.

**HLA-B27: driver of autoimmunity or autoinflammation?**

Although SpA displays many autoinflammatory features, it has originally been classified as a ‘mixed’ disease, due to the strong genetic association with HLA-B27, which suggested a pathogenic involvement of autoreactive CD8+ T cells. HLA-B27 accounts for circa 40% of the genetic risk and its direct etiopathogenetic role has been demonstrated by the development of experimental spondyloarthritis in HLA-B27/hβ2m transgenic rats [20]. As MHC class I molecule, the main function of HLA-B27 is to present antigens to CD8+ T lymphocytes after forming a complex with beta-2 microglobulin (β2m). In this context, HLA-B27 was

| Table 1. Overview of the main features of autoinflammatory and autoimmune diseases. |
|-----------------------------------------------|-----------------------------------------------|
| Autoinflammatory diseases | Autoimmune diseases |
| Immune system | Innate immunity | Adaptive immunity |
| Clinical features | Recurrent attacks | Continuous progression |
| Local triggers | |
| Laboratory findings | No autoantibodies | Autoantibodies |
| Genetic susceptibility | Monogenic > Polygenic | Polygenic > Monogenic |
| Genes involved in cytokine pathways and pathogen recognition | |
| Therapy | Anti-neutrophil | Anti-B cell |
| Anti-cytokine | Anti-T cell |
| Hypothesis | The “danger” model (Matzinger) | The “self/nonself” discrimination model |
| Examples | Monogenic: FMF, TRAPS, PAPA, CMRO | Polygenic: RA, SLE, type I diabetes mellitus, Hashimoto thyroiditis, MG, coeliac disease |
| Polygenic: Crohn’s disease, gout, Behcet’s disease | Monogenic: APS-1, IPEX, ALPS |

Table 1. Overview of the main features of autoinflammatory and autoimmune diseases.

FMF = familial Mediterranean fever, TRAPS = TNF receptor associated periodic syndrome, PAPA = pyogenic arthritis, pyoderma gangrenosum and acne syndrome, CMRO = chronic multifocal recurrent osteomyelitis, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, MG = myasthenia gravis, APS-1 = autoimmune polyendocrine syndrome-1, IPEX = immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, ALPS = autoimmune lymphoproliferative syndrome.
hypothesized to present specific bacterial peptides and thereby activate cytotoxic T cells, which could subsequently cross-react with self-peptides [21]. The “arthritogenic peptide” concept, however, was severely challenged by two studies [22,23] indicating that the absence of functional CD8+ T cells does not prevent disease in the HLA-B27/β2m rat model. More recently, two alternative hypotheses were proposed to explain the role of HLA-B27 in SpA pathogenesis (Figure 1). Both hypotheses are independent of antigen presentation, but related to intrinsic biochemical properties of the cysteine residue in the beta-pocket of HLA-B27. The first hypothesis follows the observation that HLA-B27 has the tendency to form homodimers on the cell surface [24]. HLA-B27 dimers are recognized by specific killer

<table>
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<th>Drug</th>
<th>Mechanism of action</th>
<th>Study type</th>
<th>SpA sub-type</th>
<th>Study design</th>
<th>Primary endpoint</th>
<th>Main result</th>
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| Abatacept| Inhibits T cell co-stimulation | Open-label | AS           | 1. Anti-TNF naïve (n=15)  
2. Anti-TNF failure (n=15) | ASAS40 at week 24 | 1. 13%  
2. 0% | [15] |
| Abatacept| Inhibits T cell co-stimulation | RDBPC      | PsA          | 1. Placebo (n=42)  
2. Abatacept 3 mg/kg (n=45)  
3. Abatacept 10 mg/kg (n=40)  
4. Abatacept 30/10 mg/kg (n=43) | ACR20 at day 169 | 1. 19%  
2. 33%  
3. 48%  
4. 42% | [9] |
| Alefacept| Inhibits T cell activation | RDBPC      | PsA          | 1. Placebo + MTX (n=62)  
2. Alefacept + MTX (n=123) | ACR20 at week 24 | 1. 23%  
2. 54% | [10] |
| Alefacept| Inhibits T cell activation | Open-label | PsA          | Alefacept + MTX (n=160) | ACR20 at week 24 | 54% | [11] |
| Efalizumab| Inhibits T cell activation | RDBPC      | PsA          | 1. Placebo (n=53)  
2. Efalizumab (n=54) | ACR20 at week 12 | 1. 19%  
2. 28% | [13] |
| Rituximab| B cell depletion      | Open-label | AS           | 1. Anti-TNF naïve (n=10)  
2. Anti-TNF failure (n=10) | ASAS20 at week 24 | 1. 50%  
2. 30% | [14] |
| Rituximab| B cell depletion      | Open-label | PsA          | 1. Anti-TNF naïve (n=6)  
2. Anti-TNF failure (n=15) | ACR20 at week 24 | 1. 50%  
2. 29% | [16] |

Table 2. Summary of the clinical trials with B and T cell targeted therapies in SpA. RDBPC = randomized double-blind placebo-controlled
cell immunoglobulin-like receptors (KIRs) and leukocyte immunoglobulin-like receptors (LILRs) on NK and T cells, leading to cell activation and production of pro-inflammatory mediators [25]. Unfortunately, this hypothesis is not able to explain why other HLA-B molecules or HLA-B27 subtypes, which also form homodimers, are not associated with SpA [*26,27]. The second hypothesis is based on the tendency of HLA-B27 to misfold inside the endoplasmic reticulum (ER) and generate a so-called unfolded protein response (UPR) [*28,29]. The UPR leads to the induction of ER chaperones and the activation of NF-κB, followed by increased production of pro-inflammatory cytokines. ER stress as a result of chemical agents or HLA-B27 up-regulation was especially shown to increase the TLR-induced IL-23 production by bone marrow-derived macrophages (BMDMs) from HLA-B27/Huβ2m transgenic rats [30]. ER stress in human dendritic cells (DCs) was also shown to preferentially increase the production of IL-23 after TLR stimulation [*31]. Circumstantial evidence that HLA-B27-induced UPR may be important in SpA is provided by the facts that IL-23 is highly expressed in the bowel of HLA-B27/huβ,m transgenic rats [30], as well as Crohn and SpA patients [32], and that inhibition of the IL-23p40 unit showed a good clinical efficacy in SpA-related diseases, such as Crohn’s disease, psoriasis, and psoriatic arthritis (PsA) [33-36]. It has to be noted, however, that overexpression of the β2m in the HLA-B27/hβ2m rat model in order to reduce the UPR did not prevent, but rather modulated the disease phenotype [37]. Additionally, it was recently reported that the increased LPS-induced IL-23 production by monocyte-derived macrophages of ankylosing spondylitis (AS) patients in comparison with healthy donors is not associated with the induction of an UPR, even after up-regulation of HLA-B [*38]. Thus, these two studies question the in vivo relevance of HLA-B27-induced UPR in SpA.

Although the relative contribution of each of these pathways (antigen presentation, homodimer formation and endoplasmic misfolding) to the pathology remains to be fully determined, an autoinflammatory role for HLA-B27 is further supported by the importance of tissue stress as trigger for SpA. Tissue stress is indicated by the typical localization of the lesions at sites of high mechanical stress (entheses, spine, and the large joints of the lower limbs) and by the association between SpA and bacterial infections. The latter is clinically evidenced by the development of reactive arthritis after gastrointestinal or genito-urethral infections and by the absence of disease in HLA-B27/hβ2m rats which were kept in germ-free conditions [39]. In addition, HLA-B27 expressing human monocytic cells were less able to clear Salmonella infection [40] and were more permissive for intracellular Salmonella replication, due to HLA-B27 misfolding [41]. Taken together, these findings support the hypothesis that HLA-B27 in conjunction with mechanical or bacterial stress leads to abnormal innate immune responses.
Macrophage polarization

As HLA-B27 modifies the innate immune responses of myeloid cells in general and macrophages in particular, it is crucial to understand the phenotypical and functional heterogeneity of macrophages in chronic inflammation. During their maturation process, macrophages can be polarized by local mediators into distinct functional subsets. Initially, two main macrophage types were described based on their pro- versus anti-inflammatory functions. Classically activated macrophages (M1) are specialized in the clearance of intracellular pathogens, while alternatively activated macrophages (M2) have immunoregulatory properties and are involved in scavenging debris, angiogenesis, and
tissue repair. IFN-γ is the prototypic inducer of M1, whereas numerous stimuli such as IL-4, IL-10, and costimulation with ICs and TLR ligands, are described to induce different M2 subsets [42]. Studying the phenotype and function of macrophages in vivo has proven to be a major challenge, since macrophages are a highly heterogeneous and dynamic cell population. Three issues arise from the effort to validate reliable markers, in order to identify distinct macrophage subsets in vivo. Firstly, extrapolation of data from animal models into humans should be done carefully, due to important interspecies differences. Secondly, it needs to be assessed whether in vitro polarized macrophages are a good model for the in vivo polarization. Finally, it is largely unknown whether a certain macrophage phenotype can be associated with a unique functional profile, or whether distinct phenotypic subsets display overlapping functions.

Macrophage polarization in spondyloarthritis

Immunohistologic analysis of the synovial tissue in chronic inflammatory arthritis showed a predominant infiltration with innate immune cells, and especially macrophages. Tissue macrophages are described to consist of resident macrophages and monocytes which are recruited in inflammatory conditions and differentiate upon tissue entry. The number of macrophages in the inflamed synovium was shown to correlate with the disease activity [43,44] and to decrease after clinically efficient treatment in both RA [45] and SpA [46,47]. Interestingly, despite similar numbers of synovial macrophages and overall levels of synovial inflammation, the expression of CD163, which is a known marker for M2 macrophages, was repeatedly found to be significantly increased in SpA compared to RA synovitis [44,48-53]. The expression of CD163 was also increased in the colonic mucosa of SpA [48] and Crohn’s patients versus colitis ulcerosa patients and healthy controls [54], illustrating the link between the two diseases. Furthermore, gene expression analysis of monocyte-derived myeloid cells revealed a “reversed” IFN-γ signature in human SpA [55] as well as in HLA-B27 transgenic rats [56]. Taken together, these data suggest a preferential M2 over M1 polarization in SpA, but formal proof of this hypothesis is still awaited.

Macrophage function in spondyloarthritis

Although the functional consequences of this preferential M2 polarization remain speculative, a direct link to disease pathogenesis was suggested by the correlation of the number of CD163+ synovial macrophages with disease activity and HLA-B27 positivity in SpA [49]. In agreement with the reported anti-inflammatory function of soluble CD163 [57], CD163 expression was also associated with impaired lymphocyte activation in SpA synovitis [49]. Such an immunomodulating effect of M2 macrophages may relate to disease
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pathogenesis, as suggested by the intracellular persistence of Chlamydia, which is also a causative agent for reactive arthritis, after alternative macrophage polarization in the presence of IL-4 [58].

Analysis of synovial fluid [59] further supported the idea of a shift in the M1/M2 balance as, despite comparable degrees of overall synovial inflammation and IL-6 levels, the M1 pro-inflammatory cytokines TNF, IL-1 and IL-12p40 were relatively decreased in SpA versus RA. TNF, IL-1 and, as previously discussed, IL-23 are the main macrophage-derived proinflammatory cytokines of interest in the pathophysiology of SpA. The pathogenic role of TNF is evident as TNF blockade resulted in a significant decrease in disease activity [*60], accompanied by a rapid decrease in the number of synovial macrophages, PMNs, and T cells [46;47;61]. Additional arguments for the role of TNF in SpA are the genetic susceptibility conferred by TNF-R1 and TRADD [6,7] and the TNF-R1-dependent arthritis and colitis in TNF(Delta)ARE mice [62]. Despite this overwhelming evidence for a pathogenic role of TNF, it remains poorly understood which form of TNF (soluble or transmembrane), which receptor (TNFR1 or TNFR2) and which target cells are predominantly involved in human SpA. In Crohn’s disease, which shows a broad clinical and pathogenic overlap with SpA, decreased TNF levels as a result of enhanced lysosomal degradation were suggested to be responsible for defective bacterial clearance [**63]. Moreover, overexpression of soluble TNF in mice leads to a severe and destructive polysynovitis reminiscent of human RA [64], whereas transmembrane TNF transgenic mice develop axial en peripheral joint involvement resembling human SpA [65]. Further investigation of TNF biology in human SpA remains thus warranted to define its exact pathophysiological role.

In contrast to TNF, only few data are available on IL-1 in SpA. A role for this second major macrophage-derived pro-inflammatory cytokine is again suggested by genetic polymorphisms in genes related to the IL-1 pathway [8]. However, biological investigation of IL-1 in SpA is lacking and proof-of-concept trials with the soluble IL-1R antagonist anakinra failed to consistently show clinical efficacy [66].

Besides the major pro-inflammatory cytokines (TNF, IL-1, and IL-23), macrophages are also the major source of IL-10. This prototypical immunoregulatory cytokine was shown to play a key role in the maintenance of immunological tolerance and is mainly produced by M2 macrophages. Dysregulation of the IL-10 pathway was initially reported in Crohn’s disease [67,68] and genetic defects in the IL-10R lead to severe juvenile-onset colitis [69]. Whether the IL-10 pathways is also dysregulated in SpA remains to be investigated in more detail, but the reduced constitutive and bacterial-induced IL-10 production by splenocytes from HLA-B27/hβ2m rats [70] and reduced IL-10 production by AS PBMCs after stimulation with autologous commensal enteric bacteria [71] are compatible with this hypothesis.
The role of other innate immune cells in spondyloarthritis

As discussed, functional and genetic data suggest an essential role for the IL-23/STAT3 pathway in SpA. Polymorphisms in the IL-23R and STAT3 genes are associated with disease predisposition in SpA, as well as IBD [72,73]. Moreover, blockade of IL-17A, an effector cytokine triggered by IL-23/STAT3 signaling, showed good clinical efficacy in a proof-of-concept clinical trial in AS [*74]. Whereas the IL-23/IL-17 axis has mainly been studied in canonical Th17 cells, the data on the number of Th17 cells in peripheral blood of SpA remain inconsistent [75,76]. More importantly, direct analysis of the axial and peripheral joints of SpA patients showed that IL-17 was not expressed by T cells, but rather by innate immune cells, including mast cells (MCs) and neutrophils [53,**77,**78,*79](Figure 2)[80-82,*83,*84]. Accordingly, targeting MCs with the c-kit inhibitor imatinib induced a marked decrease of IL-17 production by SpA synovial biopsies [53]. These data are consistent with the fact that IL-23 was shown to trigger IL-17 production not only by Th17 cells, but also by γδ T cells [*83,85], innate lymphoid cells (ILC), which are the main producers of IL-17 in experimental colitis [*84,*86], MCs and neutrophils, but potentially also NK cells, which can be directly stimulated by HLA-B27 homodimers. The exact role and contribution of these different cell types to the pathophysiology of SpA remains to be investigated in detail.

CONCLUSIONS

The genetic risk factors related to the innate cell biology and the predominance of innate versus adaptive immune responses in SpA indicate a prominent autoinflammatory component, according to McGonagle’s classification [2]. The importance of the intrinsic, rather than the antigen-presenting properties of HLA-B27 further suggests that SpA belongs to the same class of polygenic autoinflammatory disorders as Crohn’s and Behcet’s disease. However, as innate and adaptive immune responses are intimately entangled and chronic tissue inflammation is often accompanied by secondary T and B cell activation, a dichotomous classification might prove too simplistic. The major challenge is to define the exact phenotype and function of innate immune cells, focusing on the target tissues, and to relate this biologically to the genetic and environmental factors involved in disease pathogenesis. Better understanding of the innate immune alterations in SpA may help to fine tune anti-cytokine therapies. It may also lead to new therapeutic strategies, such as direct targeting of specific innate immune cells, or upregulation of innate immunoregulatory programs, which may hold promise of longer term efficacy in comparison with anti-cytokine therapy.
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**Key points**

- SpA does not display the prototypical genetic, clinical and immunological features of T and/or B cell mediated autoimmune diseases.
- B and T cell targeted therapies are not effective in SpA.
- HLA-B27-dependent UPR leads to augmented IL-23 production, but the role of UPR in vivo remains to be investigated in more detail.
- Alternative macrophage polarization is a hallmark of SpA.
- Innate immune cells rather than T cells are the main producers of IL-17 in SpA.

**Figure 2. Overview of the IL-23-responsive immune cells which are involved in the pathogenesis of human IBD and SpA.** Th17 cells were shown to play a pathogenetic role in IBD, where they can exert both inflammatory and immunosuppressive effects [80,81], but there are indications that they are also involved in axial SpA [82]. IL-23R+ γδ T cells were found to be increased in peripheral blood of AS and PsA patients compared to RA patients and healthy individuals [*83]. IL-23 was further shown to control the function of innate lymphoid cells (ILCs), which were selectively increased in the inflamed intestine of Crohn’s disease, but not ulcerative colitis patients [*84]. Other innate immune cells which were found to be increased in both axial and peripheral SpA are neutrophils [**78,*79] and mast cells (MCs) [53,**77,*79].
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References
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   This review discusses the most recent findings concerning the genetic risk in ankylosing spondylitis.
   This review discusses the role of HLA-B27-induced unfolded protein response in the pathogenesis of spondyloarthritis.
   This study demonstrates that IL-23 production by human dendritic cells after ER stress and TLR stimulation is downstream of the CHOP transcription factor.
34. Leonardi CL, Kimball AB, Papp KA, et al. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a
** This paper shows a dramatic LPS-induced increase in IL-23 production by ankylosing spondylitis versus healthy macrophages, without significant changes of ER stress markers.**  


69. Glocker EO, Kotlarz D, Boztug K, et al. Inflammatory bowel disease and mutations affecting the

This important clinical trial shows good efficacy of IL-17 blockade in ankylosing spondylitis patients.


These three studies, along with that published by Noordenbos et al [53], demonstrate that innate immune cells such as mast cells and neutrophils express IL-17 in both axial and peripheral synovitis and play an important role in the pathogenesis of spondyloarthritis.


This study demonstrates that IL-23 drives IL-17 production by γδ T cells, which are possibly involved in the pathogenesis of ankylosing spondylitis.


This study shows that IL-23-responsive innate lymphoid cells are selectively increased in the inflamed intestine of Crohn’s disease patients.

This study describes previously unidentified innate lymphoid cells which respond to IL-23 and mediate intestinal inflammation.