AXIAL SPONDYLOARTHRITIS – FROM INFLAMMATION TO ANKYLOSIS

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Abstract. Axial spondyloarthritis is a chronic inflammatory immune-mediated rheumatic disease that mainly affects the sacroiliac joints and the spine and encompasses both sub-units – ankylosing spondylitis and its preceding phase non-radiographic axial spondyloarthritis. The disease is characterized by two main immunopathological processes – chronic inflammation and pathological new bone formation, the causal relationship of which is still not fully understood. Starting as enthesopathic inflammation in the early stages, the disease progresses to ossifying enthesitis as a result of an abnormal immune response to skeletal biomechanical stress associated recurrent tissue microdamage, and a subsequent process of excessive repair and tissue remodeling. Immune-mediated inflammation manifests with a distinct skewing of differentiation towards a Th1/Th17 phenotype and an unbalanced profile of cytokine production, with cytokine dysregulation and predominance of the effects of pro-inflammatory cytokines. Molecular signaling pathways of syndesmophyte formation include bone morphogenetic protein (BMP), wingless-type like (WNT), Dickkopf-1 (Dkk-1), sclerostin, cytokines, and others. The review summarizes the current concepts regarding the pathophysiology of both pathognomonic processes for the disease – inflammation and pronounced osteoproliferation.

Key words: axial spondyloarthritis, ankylosing spondylitis, inflammation, new bone formation

INTRODUCTION

Axial Spondyloarthritis (axSpA) is a chronic, inflammatory immune-mediated rheumatic disease that affects primarily the axial skeleton (the sacroiliac joints and the spine), but is also characterized by other musculoskeletal and extra-musculoskeletal pathological manifestations. Ankylosing spondylitis (AS), also called radiographic axial spondyloarthritis (r-axSpA) is the terminal, more severe phenotype of axSpA while its earlier phase until the formation of radiographic sacroiliitis which could be visualized only by magnetic resonance imaging (MRI), is termed as non-radiographic axial spondyloarthritis (nr-axSpA). These are two distinct conditions within the scope of the same disease process but not different disease entities [1]. Two main immunopathological processes emerge in the pathophysiology – chronic inflammation and pathological new bone formation – and they are pathognomonic for the disease. Whether inflammation and ankylosis are interrelated and mutually conditioned has been discussed for a long time [2] as the prevailing views are in favor of a link between them.

IMMUNE-MEDIATED INFLAMMATION AND CYTOKINE DYSREGULATION IN THE PATHOGENESIS OF THE axSpA. TH17 IMMUNE RESPONSES AND IL-17/23 IMMUNE AXIS

SpA and, in particular AS, as a prototypical disease from the group of spondyloarthritis, is a pathology that is situated in the spectrum of immune-mediated diseases, occupying an intermediate place between autoimmunity and the autoinflammatory process [3]. According to the current concept, it is driven to a greater extent by the subset of T helper (Th) 17 lymphocytes, producing IL-17A (as well as other pro-inflammatory cytokines), which stimulate signals characteristic of early inflammatory processes and thus serve as a bridge between acquired and innate immunity [4]. The loss of the balance between Th17/Treg cells toward increased regulation of Th17 is considered responsible for the breakdown of immunological tolerance and the development of autoimmune and chronic inflammatory diseases [5], including AS. Numerous other cytokines with predominantly pro-inflammatory effects, well-known key
mediators of chronic tissue inflammation, such as T helper subtype 1 (Th1) and macrophage cytokine TNF-α and IL-6, which is produced by many different cell types, are involved in the pathogenesis of AS. There is a distinct bias of differentiation towards the Th1/Th17 phenotype in the immunological inflammatory basis of the disease and an unbalanced profile of cytokine production, with cytokine dysregulation and a predominance of the effects of pro-inflammatory cytokines over those of anti-inflammatory ones [6-8] (fig. 1).

Fig. 1. Skewing differentiation towards Th1/Th17 immune responses

Enriching knowledge about the pathophysiology of immune-mediated diseases such as AS has led to repeated emphasis on the key role of cytokine dysregulation. This knowledge has served for the development of innovative targeted therapeutic strategies directed at the key cytokines in the immunoinflammatory process. Although cytokine molecules are mediators of normal immune function and an integral part of the immune defense network, imbalances in their levels could cause both acute and chronic inflammatory disorders [9], with the latter being the leading ones in this case. Th17 immune responses are characterized by the production of IL-17 (as well as other pro-inflammatory cytokines – IL-17F, IL-22, GM-CSF and IL-9) and depend to a significant extent on IL-23, which is responsible for expanding and maintenance of their phenotype, underlying the concept of the IL-17/23 immune axis. In addition to the IL-23-dependent pathway involving the acquired immune response, in which TNF-α-activated dendritic cells produce IL-23 as the main stimulator of Th17 lymphocytes for IL-17 secretion [10], another IL-23-independent pathway exists in which the secretion of IL-17A is realized by multiple cell lines of innate immune activation – macrophages, mast cells and neutrophils, as an early source of IL-17 in response to pathogenic influence [11]. The pathogenetic role of IL-17A for AS downstream most likely results from its effect on the recruitment of neutrophils, macrophages and epithelial cells and subsequent release of other pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α [12].

TNF-α is also a cytokine with proven contribution in the pathogenesis of AS, whose important role is also confirmed by the successful treatment of the disease with anti-TNF drugs. It is a highly potent pro-inflammatory molecule and a key signaling component of the immune system [13]. It has a significant contribution to both the acute phase response and systemic inflammation and has an extremely wide range of biological activities. It is considered a key mediator of inflammation, by activating leukocytes, enhancing the adhesion of neutrophils and monocytes to the endothelium, stimulating the migration of inflammatory cells into the intercellular matrix, stimulating the proliferation of fibroblasts and inducing the local production of other pro-inflammatory cytokines [14]. Upon neutralization of TNF-α, inhibition of IL-1 and a number of other pro-inflammatory cytokines occurs, supporting the notion that pro-inflammatory cytokines are connected in a network at the tip of which is TNF-α. The concept of the central role of TNF-α in many immune-mediated diseases is firmly accepted.

**Signalizing pathways for triggering of Th1 and Th17 immune responses**

Cytokines are soluble glycoproteins that act in the extracellular space as key mediators of cell signaling, inducing proliferation, differentiation, growth, or apoptosis of their target cells. For the accomplishment of these critical events in the cell, extracellular signals need to be transmitted to the nucleus for modulation of target gene transcription; hence cytokine signaling systems are composed of receptors and downstream signaling molecules. In general, cytokines trigger signaling cascades (signalizing pathways) – a series of biochemical reactions initiated...
by a primary stimulus acting on a receptor that is transmitted inside the cell via a second mediator that amplifies the initial signal – intracellular signaling molecules (kinases, phosphatases, adapters and transcription factors) and ultimately to effector molecules, resulting in a cellular response to the initial stimulus [15]. Cell signaling is part of the molecular biology system that controls and coordinates the actions of cells. In this way, the control of cell function, such as cell division or cell death, and the biological effects on various immune cells is achieved. Key cytokines involved in Th1 and Th2 cell lines trigger one of the main signaling cascades – the Janus kinase and signal transducer and activator of transcription (JAK-STAT) signaling pathways to the cell nucleus and thus exert their effects at the molecular level [16]. Further, STATs bind to DNA, enabling the transcription of target genes encoding immune cell division, survival, activation, and recruitment. Thus, the JAK/STAT cascade provides a direct mechanism for translating an extracellular signal into a transcriptional response. Unlike traditionally Th1 and Th2 cytokines that operate through the evolutionarily highly conserved JAK/STAT signaling pathway, IL-17 family cytokines mediate signaling through a novel ACT1-dependent pathway culminating in the activation of pro-inflammatory transcription factors such as NF-κB, which normally are related to innate immunity. Thus, thanks to the unusual signaling properties of IL-17, Th17 cells stimulate signals typical of early inflammatory processes and in this sense make a direct link between adaptive and innate immunity [16, 17]. Therefore recently AS is recognized as pathology triggered by innate immunity to a greater extent, unlike psoriasis, for example, in which the pathological process is driven by acquired immunity. Interestingly, another Th17 distinctive cytokine is IL-22, which activates the JAK-STAT3 signaling program, but the downstream gene targets are strikingly similar to the genes induced by IL-17 [16].

Briefly, against the background of genetic predisposition and permanently acting initiating triggers, a disordered regulation and continuous production of pro-inflammatory cytokines emerges, forming cytokine cascades [6] and contributing to strengthening of immune responses, development of immunopathological processes in response to the triggering trigger, and ultimately, failed resolution of the inflammation with sustainability of the disease. The cellular composition in AS is represented by numerous immune cells of the innate (monocytes, dendritic cells, mast cells, NK cells) and acquired immunity (Th1, Th17, B lymphocytes) and tissue response cells (endothelial cells, fibroblasts). Pathological lesions in the axial and peripheral skeleton are characterized by soft tissue inflammation and subchondral bone marrow inflammation with infiltration of CD8+ and CD4+ T cells, B cells, macrophages and osteoclasts [18].

**Enthesial inflammation as a primary pathological process underlying of axSpA**

In recent years, the prevailing hypothesis is that the primary site of inflammation in axSpA occurs at the attachment sites of the tendons or ligaments to the bone, in the area of the entheses, representing a fibrocartilaginous connection, and enthesis is the initial pathological process underlying SpA. From the point of view of the specific processes involved in predominantly enthesopathic inflammation with the development of ossifying enthesitis, AS is a quite different disease state from chronic inflammatory arthritis, occurring with predominantly synovial inflammation and appearance of destructive arthritis. The synovium and underlying bone marrow are in close contact and communication with the entheses; hence the concept of synovitis and osteitis in SpA is based on this close anatomical relationship [19-21].

According to some authors, a key event in the early stages of AS is the association with peri-fibrocartilaginous osteitis in the sacroiliac joints and enthesial attachment sites to the bone, as well as in bone areas adjacent to the synovio-enthesis fibrocartilages. Chemotaxis and accumulation of inflammatory cells coupled with increased angiogenesis are more easily accomplished in accessible synovium and bone marrow than in enthesial fibrocartilage, which is relatively resistant to cell invasion and neovascularization [20, 21]. Entheses are structures that are subjected to severe biomechanical stress, and their inflammation leads to erosions and bone proliferation through mechanisms that are poorly understood. This skeletal propensity is associated with an abnormal immune response to skeletal biomechanical stress and the associated recurrent tissue microdamage, followed by excessive reparation and tissue remodeling [19, 22]. Syndesmophytes are thought to form as a result of excessive reactions of recovery and chronic new bone formation that develops in response to inflammation and includes cartilage metaplasia with advanced AS. Subsequently, blood vessels and osteoblast precursors invade the chondrocyte matrix and replace the cartilage tissue with bone [23]. Structural damage to the skeleton is one of the factors that determine the clinical out-
come of the disease and impairments in functional capacity [24].

**Molecular mechanisms and regulation of the osteoproliferative process in axSpA**

Evaluation of the molecular mechanisms of ankylosing enthesopathy has shown that it is based on a number of molecular signaling pathways and their complex and sometimes contrasting interactions. These pathways include bone morphogenetic protein (BMP), wingless-type like (WNT), hedgehog, fibroblast growth factors, notch, and parathyroid hormone-like peptide signaling [25] (fig. 2).

**Bone morphogenetic proteins in the development of axSpA**

Bone morphogenetic proteins (BMPs) are intercellular signaling molecules, members of the transforming growth factor β (TGFβ) family, that play a critical role in the differentiation of mesenchymal cells into osteoblasts by binding to their surface receptors. They are potent stimulators of bone formation. Aforementioned potent bone-inducing activity has been confirmed for BMP2, 4, 6, 7 and 9, termed as osteogenic BMPs [26]. Although the name suggests that all members are bone inducers, some BMPs act as inhibitors of bone formation, such as BMP3, which is a negative regulator of bone density, and BMP13, which is a strong inhibitor of bone formation [27, 28].

On the other hand, BMPs also perform other cellular functions beyond bone and osteogenesis. The function of many other organs and systems is influenced by one or more BMPs. They have an important morphogenetic role during embryogenesis and intrauterine development and regulate the maintenance of tissue homeostasis in the postnatal period in adults by mediating cell proliferation, differentiation and apoptosis. They have an important morphogenetic role during embryogenesis and intrauterine development, and regulate the maintenance of tissue homeostasis in the postnatal period in adults by mediating cell proliferation, differentiation and apoptosis. A number of processes such as maintenance of joint integrity, initiation of repair after fractures are dependent on BMP signaling [29, 30]. Evidence exist in the scientific literature for the involvement of BMP proteins in the development of the retina [31], of BMP4 in the development of limbs, with a regulating effect on the number and identity of fingers [32]. BMP8A and BMP4 are required for supporting the spermatogenesis and, together with BMP7, play a role in maintaining epididymal integrity and male reproductive health [33, 34]. BMP15 is a physiological regulator of ovarian follicular cell proliferation and/or differentiation and has a fundamental role in ovarian function and, in general, female fertility [35, 36].

The initial identification of BMPs as morphogens capable of triggering the complete cascade of enchondral ossification [37, 38], highlights their possible key role in initiating these processes not only in health but also in disease [39]. A study by Scharstuhl A. and co-authors on experimental models of osteoarthritis demonstrate the involvement of endogenous BMPs in osteophyte formation and synovial thickening [40]. Serum biomarkers are another research tool for studying the process of ankylosis. A Chinese study found increased serum levels of
BMP-2, 4, 6, and 7 in AS. The authors conclude that these bone formation signaling proteins might play an important role in the pathogenesis of abnormal bone remodeling in the disease [41]. These results are consistent with earlier reports of increased production of BMP-2, BMP-4 and BMP-7 [42], especially in the phase of spinal ankylosis [43], highlighting their role of significant osteoimmunological markers in the disease. Detailed histomorphological analysis reveals that BMP signaling is critically important in the early stages of the disease process, particularly in the recruitment of progenitor cells to the chondrogenic lineage [44]. Growing evidence supports the significant role of BMP signaling in joint remodeling, particularly in the enthesophyte formation in SpA.

**Role of wingless-type (WNT) signaling in axSpA**

Effects of WNT signaling on bone formation appear to be more complex. WNTs are a family of glycoproteins with a range of functions both during development, growth, tissue homeostasis and in disease [25]. Upon activation of the so-called canonical WNT signaling pathway, downstream the intracellular mediator β-catenin is activated, which translocate the signal to the nucleus. It has been reported that overexpression of the constitutively active form of β-catenin, as an expression of enhanced WNT signaling in postnatal development, stimulates chondrocyte maturation and bone formation [45]. These observations are consistent with a study revealing that the progression of BMP2-induced endochondral bone formation is a β-catenin-dependent process [46]. It is hypothesized that members of the BMP family are critical in the early phases of ankylosis in SpA and that WNT signaling through β-catenin plays a crucial supporting role in this process, particularly in the progression of endochondral bone formation [25].

Wnt signaling is regulated by various inhibitors, such as sclerostin (SOST) and the Dickkopf (Dkk) family of secreted proteins. Sclerostin is a protein that is mainly secreted by mature osteocytes and acts downstream of the Wnt/β-catenin signaling pathway by shortening the lifespan of osteoblasts by promoting their apoptosis [47]. In this regard, it should be noted that osteocytes play a role as key factors in the regulation of the canonical Wnt signaling pathway as targets of Wnt ligands and through the secretion of molecules modulating the effects of Wnt [48, 49]. A study by Appel H. et al found almost absent expression of sclerostin in osteocytes in the periarticular bone of patients with AS, suggesting a specific alteration of osteocyte function in this disease. This study showed that radiographic progression was associated with significantly lower sclerostin levels, suggesting that low sclerostin levels in AS increase the predisposition to syndesmophyte formation [50]. The same authors, as well as others, demonstrated lower serum sclerostin levels in AS patients compared to healthy controls [50 - 52].

**Dickkopf-1 (Dkk-1) and axSpA**

Dkk is a family of proteins comprising at least four different forms (Dkk-1 to Dkk-4); the most studied is Dkk-1, a potent antagonist of canonical Wnt/β-catenin signaling [53]. Besides, Dkk-1 further inhibits Wnt signaling by inducing the other antagonist sclerostin [52]. The review of the scientific literature provides divergent and conflicting data regarding circulating levels of Dkk-1 and their correlation, but nevertheless confirms its involvement in the pathogenesis of structural damage in SpA. A study conducted in Greece demonstrated that serum levels of Dkk-1 were significantly higher in patients with AS compared to those with rheumatoid arthritis and healthy controls, and this was particularly pronounced in those receiving anti-TNF treatment [54]. These results suggest that higher levels of Dkk-1 reflect counterbalancing mechanisms aiming to attenuate Wnt signaling, which unlocks after resolution of treatment-induced inflammation. In contrast, another study revealed that circulating levels of Dkk-1 were lower in AS patients compared to healthy controls and did not change after anti-TNF treatment [55]. One possible explanation for the lack of efficacy of TNF inhibitors in stopping syndesmophyte formation in SpA is based on inability of targeted therapies to increase DKK-1 and sclerostin production and suppress Wnt-mediated bone formation [56].

**IL-22 in post-inflammatory new bone formation in the entheses region in SpA**

SpAs are genetically and therapeutically linked to IL-23, which in turn upregulates IL-22, a cytokine involved in the regulation of new bone formation. Studies in mouse models have identified resident enthesial cells that respond to IL-23 and mediate spinal and peripheral inflammation. Increased expression of IL-23 leads to new enthesis bone formation and osteoblast expansion through upregulation of IL-22, which induces osteoblast-related genes in the enthesis. New periosteal bone formation is mediated both by activation of STAT3 and by increased...
expression of genes regulating bone formation, such as those of the Wnt family [57]. Furthermore, a more recent study demonstrates that IL-22 affects the function of mesenchymal stem cells, including their proliferation/migration under inflammatory conditions, and is involved in subsequent osteogenesis. These effects of IL-22 on MSC function are a new direction for investigating pathological, post-inflammatory osteogenesis in SpA [58].

**Relationship between inflammation and new bone formation in axSpA**

The typical presentation of the disease – with signs and symptoms caused by inflammation evident in the early stages and ankylosis and resulting structural damage in the later stages – suggests a chronological order of events [25]. However, it is not yet clear how the new bone formation that develops after inflammation gains autonomy and how it is triggered [59]. According to Carter et al, inflammation and ankylosis are related but largely molecularly independent processes [39]. According to findings of Lories RJ, new tissue formation and inflammation appear to be at least partially unrelated events. Different pathways regulate chronic inflammation and new tissue formation, but these pathways are likely to influence each other [25, 44].

The study of the relationship between the two pathological processes is primarily based on imaging studies – MRI for imaging active inflammatory lesions and conventional radiography, as the gold standard for visualizing structural changes. Results from prospective studies support the conception that new bone formation follows the process of resolution of inflammatory lesions and their replacement by fat and that focal fatty infiltration at the vertebral angles is associated with the development of new syndesmophytes after 2 years [59].

Analyzes of spinal MRI images and conventional radiographs in AS patients treated with anti-TNF agents at baseline and after 2 years showed that although syndesmophytes developed at sites where no MRI inflammation was observed at initiation, they are more likely to develop in the areas with inflamed vertebral angles. These data suggest both an association and some dissociation of inflammation and new bone formation in AS [60]. In contrast to previously published results from shorter, two-year studies of the lack of effect on structural progression in AS with anti-TNF-α treatment [61, 62], more recent analyzes showed a reduction in radiographic progression of spinal changes after more than 4 years of follow-up in AS patients receiving long-term therapy with TNF inhibitors [63]. These data highlight the importance of timely administration of anti-TNF-α therapies, blocking the post-inflammatory fatty transformation of subchondral bone marrow edema and preventing the development of fatty infiltration in the bone marrow after inflammation, which is associated with a worse prognostic phenotype characterized by increased propensity for new bone formation in both SI joints and the spine [59]. Since anti-TNF-α treatment affects areas with incipient new inflammation, without fatty transformation, and does not stop progression in areas where fatty infiltration is already present, long-term use of these medications is only able to slow but not completely stop the formation of new bone [59, 60]. For these reasons, both the timely and early suppression of inflammation and the search for potential therapeutic targets directed against the formation of enthesophytes in SpA are essential.

In conclusion, the relationship between inflammation and syndesmophyte formation is still not fully understood. Further study of the link and pathological progression from inflammation to bone formation is needed.

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