Emerging concepts in ankylosing spondylitis

Ankylosing spondylitis (AS) is a chronic disease characterized by inflammatory back pain and progressive stiffening of the spine. The association between AS and HLA-B27, a major histocompatibility complex class I molecule, has been known since 1973. Despite intense interest and active investigations into the role of HLA-B27 in the pathogenesis of AS, the specific role of HLA-B27 in AS is not yet known. One of the postulated hypotheses states that misfolding of the HLA-B27 heavy chains causes activation of the unfolded protein response, which sensitizes cells to proinflammatory cytokine production. The IL-23/Th17 axis may play an important role in this pathway. As AS is a multifactorial disease, not only genetic factors are of interest, but environmental factors, such as biomechanical stress, may also play an important role in the pathogenesis. None of the postulated mechanisms regarding the pathogenesis of AS have been strictly proven, but current results from studies are promising.

KEYWORDS: ankylosing spondylitis ■ biomechanics ■ endoplasmic reticulum stress ■ IL-23 cells ■ pathogenesis ■ Th17 cells ■ unfolded protein response

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Ankylosing spondylitis (AS) is a common chronic disease characterized by inflammatory back pain and progressive stiffening of the spine. It is the major subtype of spondyloarthritis, a group of inter-related and overlapping chronic inflammatory rheumatic diseases [1,2]. Besides AS, this disease concept consists of psoriatic arthritis, reactive arthritis, arthritis related to inflammatory bowel disease, juvenile idiopathic arthritis and undifferentiated spondyloarthritis [1,3]. Identifying a clear difference among the different forms is not always possible as clinical features overlap and the dominant disease presentation may vary over time [4].

In western Europe, the prevalence of AS is estimated to be between 0.3 and 0.5%. For the group of spondyloarthritis it is 1–2%, which is similar to that of rheumatoid arthritis. AS affects young people with common onset in the late second and early third decade of life [1]. Initially, AS was thought to be an overwhelmingly male disease with only a few women affected; however, the male:female ratio is currently estimated at 2:1 to 3:1 [5].

The main clinical features of spondyloarthritis are inflammatory back pain caused by sacroiliitis and spondylitis, peripheral arthritis, enthesitis, dactylitis and extra-articular manifestations such as anterior uveitis, inflammatory bowel disease and psoriasis [1]. Characteristics of inflammatory back pain have an age at onset of <40 years, insidious onset evolving over weeks or months, improvement of pain and stiffness upon exercise, no improvement with rest and pain at night (with improvement upon getting up) [6].

The characteristic symptoms of AS in particular are spinal stiffness and loss of spinal mobility [1]. This impairment of spinal mobility is determined by reversible spinal inflammation, as well as by irreversible spinal damage. The first will dominate in early disease, whereas the latter may become the dominant determinant in later disease [7]. Structural damage in AS is due to new cartilage and bone formation, leading to formation of bony spurs, syndesmophytes, enthesophytes and finally sacroiliac joint or spine ankylosis [8].

Currently, NSAIDs remain the cornerstone of the treatment of AS. Numerous studies have demonstrated that NSAIDs provide a rapid relief of inflammatory back pain and stiffness and an improvement of physical function [9,10]. Moreover, studies demonstrate decreased radiographic progression in AS patients when treated with a continuous high dose of NSAIDs [11,12]. Clinical experience also shows benefit from intensive physiotherapy in individual AS patients, but the general effect size at the population level of nonpharmacological intervention is rather small [1]. There is no evidence for the use of disease-modifying antirheumatic drugs (sulfasalazine, methotrexate and leflunomide) and systemic glucocorticoids in the treatment of axial disease in spondyloarthritis [1,13]; however, such immune modulators may be considered in spondyloarthritis patients with peripheral

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arthritis [13]. The introduction of TNF blockers has been the most substantial development in the treatment of AS and other subtypes of spondyloarthritis in the last decade [1]. TNF blockers have a strong clinical efficacy in the short and intermediate term [14–18]. Unlike NSAIDs, however, TNF blockers do not appear to inhibit progression of structural damage in patients with AS [14,19].

With regard to the pathogenesis of AS, different hypotheses have been put forward, but the actual cause is not yet known. This review will give an overview of current concepts in the pathogenesis of AS, with special attention to the role of endoplasmic reticulum (ER) stress and biomechanical strain.

Current concepts of AS

The actual cause of AS, and of spondyloarthritis as a group, is unknown. Since the majority of the inflammatory lesions (e.g., sacroiliac joints and vertebral bodies) in AS are poorly accessible, information on histopathology is limited, which is an important limiting factor in unraveling the pathogenesis [20]. The few histological studies available, with samples from the zygapophyseal, hip and sacroiliac joints of AS patients, show infiltrates of T cells, B cells, cells involved in neoangiogenesis and bone marrow-derived macrophages and osteoclasts in the affected joints and bones [21–24].

Studies of disease concordance in twins and relatives of patients with AS have shown that the susceptibility to the disease is largely due to genetic factors [25]. The association of AS with *HLA-B27*, a MHC class I antigen molecule, has been known for approximately 40 years [26,27] and is among the strongest genetic associations with any common disease [28,29]. Nonetheless, family studies suggest that <50% of the overall genetic risk is due to *HLA-B27* [28,30]. Moreover, less than 5% of *HLA-B27*-positive people in the general population develop AS, whereas 20% of the *HLA-B27*-positive relatives of AS patients develop spondyloarthritis [25], suggesting the involvement of other genetic factors in the pathogenesis of AS. The most likely genetic model for AS is therefore oligogenic [31]. Proposed additional susceptibility genes based on large genome-wide association studies are *ERAP1*, *IL-23R*, *IL1R2*, *ANTXR2*, *TNFR1*, *STAT3*, *CARD9*, *IL1A* and gene deserts at chromosome 2p15, 21q22 and 1p32 [3,25,30]. Despite intense interest and active investigations into the role of *HLA-B27* in the pathogenesis of AS over the previous 40 years, the specific association between HLA-B27 and AS is not yet known. There are many postulated mechanisms and none of them have yet been proven or eliminated entirely [32].

The potential effect of HLA-B27

HLA-B27 is a MHC class I molecule. MHC I molecules are expressed ubiquitously and their expression is strongly upregulated by pro-inflammatory stimuli [20]. The function of the MHC I molecules is to bind peptides and display them on the cell surface where they can be recognized by CD8-positive T cells [20]. MHC I molecules consist of a polymorphic MHC-encoded heavy chain, noncovalently bound by a soluble nonpolymorphic light chain, called β2-microglobulin (β2m) (Figure 1) [33]. β2m helps to maintain the heavy chain in its proper conformation [20]. The distal domains of the heavy chain form a peptide-binding groove. The region of the peptide-binding groove that has the most dominant effect on the peptide selection is called the B-pocket [34]. When a peptide is bound, this bound peptide is an integral part of the MHC I structure [33] and presented to T cells. The assembly and folding of the complex and the loading with a peptide take place in the ER. High stability of this complex is critical for efficient transport through the Golgi apparatus towards the cell surface [32].

Approximately 90% of patients with AS are *HLA-B27* positive [35]. On the other hand, 8% of healthy Caucasians are also *HLA-B27* positive and more than 90% of them will never develop AS [2]. These epidemiologic data suggest a primary role for *HLA-B27* in the pathogenesis of AS, but does not explain why the majority of *HLA-B27*-positive individuals remain healthy [35].

Hammer *et al.*, in the early 1990s, showed that *HLA-B27*/human *β2m* transgenic rats spontaneously develop a spondyloarthritis-like phenotype, including arthritis and colitis [36]. The degree of susceptibility for spondyloarthritis-like lesions in these animals correlates with the level of *HLA-B27* transgene expression at the mRNA and protein levels, as a kind of 'threshold' effect, where a certain level of *HLA-B27*/human *β2m* expression must be achieved to obtain a disease phenotype [37]. A similar phenomenon is seen in humans, as the level of *HLA-B27* expression on peripheral blood mononuclear cells appears to be associated with the susceptibility of AS, but not with disease outcome or extra-articular manifestations [38]. The expression of *HLA-B27* in patients with AS also appears to be higher compared with

the expression in *HLA-B27*-positive healthy controls or family members [35,39,40], with no difference in expression of total MHC class I molecules between these groups [35].

In the *HLA-B27*/human *β2m* transgenic rats, several lines of evidence strongly support an important role for dendritic cells in the pathogenesis. Multiple studies show a decreased stimulation of T cells by dendritic cells in these rats. This dysfunction precedes the disease onset, as it is also seen in premorbid and nude transgenic rats (which are protected from disease), and is therefore not secondary to chronic inflammation but may be directly linked to the presence of the *HLA-B27* transgene [41–44]. There is some evidence that this dendritic cell dysfunction is not only present in transgenicrats, but also in AS patients [45,46]. The molecular basis for the dendritic cell dysfunction by the *HLA-B27*/human *β2m* transgenes remains an important area of research.

The *HLA-B27* family consists of multiple, closely related alleles, known as subtypes [20]. The most common subtypes are *HLA-B2705* (Caucasians and American–Indians), *HLA-B2704* (Asians) and *HLA-B2702* (Mediterranean populations), all of which have a strong association with AS [45]. *HLA-B2706* and *HLA-B2709* are thought to lack association with AS, although some cases in which patients with these subtypes suffer from AS have been reported [32,47]. Many *HLA-B27* subtypes are only reported in a small number of individuals. Therefore, the prevalence of each subtype and whether some represent mutations rather than polymorphisms are not completely clear. Furthermore, because of the small reported number of some subtypes, they also lack a definitive disease association [48].

Three different hypotheses are proposed that link *HLA-B27* to AS: the arthritogenic peptide hypothesis, the free heavy-chain hypothesis and the ER stress hypothesis (FIGURE 2).

Arthritogenic peptide hypothesis

The arthritogenic peptide hypothesis, also called the molecular mimicry hypothesis, is one of the traditional pathophysiological frameworks. The classical function of MHC class I proteins is to present peptides to activate antigen-recognizing CD8-positive T cells. Normally, the host CD8-positive T cells are tolerant to HLA-B27 presenting self-peptides, but can develop an adaptive immune response to microbial peptides. This hypothesis proposes that tolerance to a self-peptide can be lost if an infectious pathogen activates the immune system through a pathogen-derived peptide that mimics the self-peptide [49]. This self-peptide will then become the target of autoreactive CD8-positive T cells [3,20], which causes cytotoxicity and chronic inflammation [20]. Evidence for this hypothesis is provided by the observation that spondyloarthritis (i.e., reactive arthritis) can be triggered by gastrointestinal and urogenital infections. On the other hand, an argument against this hypothesis is the fact that disease manifestations arise in *HLA-B27*-transgenic rats in the absence of any functional CD8-positive T cells [50]. However, T cells are required for disease, as *HLA-B27*-transgenic nude rats, which lack a thymus and hence T cells, remain healthy [51].

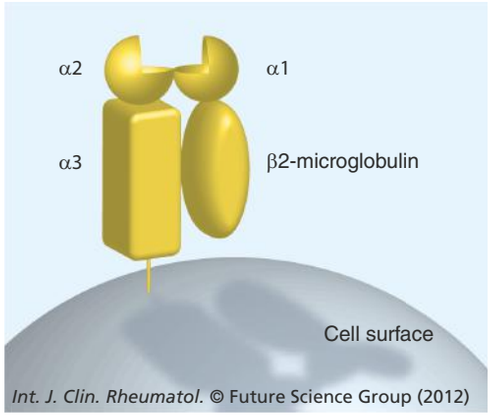

Figure 1. MHC class I molecule such as HLA-B27 at the cell surface.

Free heavy-chain hypothesis

On the cell surface, β2m may dissociate from the HLA heavy chain [49]. The free heavy chains can form disulfide-linked dimers through the unpaired Cys67 residue [20]. These dimers engage various receptors, including killer cell immunoglobulin-like receptors (KIRs) and leukocyte immunoglobulin-like receptors (LILRs) [52], on certain NK cells and T lymphocytes [49], which leads to modulation of leukocyte function and eventually the development of inflammatory disease [20]. Disulfide-linked HLA-B27 heavy-chain dimers are described intracellularly and on the cell surface, both in AS patients and in *HLA-B27*-transgenic animals [53,54]. The cell surface dimers are 'well-folded' heavy-chain dimers and are observed forming in a post-Golgi compartment [34]. It is important to make a distinction between dimer formation in the ER and in distal compartments of the cell. The former will generate ER stress (see below), while the latter will be recognized by cells of the immune system, as described above in this section [34].

ER stress & the unfolded protein response

Protein misfolding

HLA-B27 heavy chains have a tendency to misfold in the ER prior to assembly into complexes with peptide and β2m [54]. The combination of delayed heavy-chain folding and ER retention, together with formation of aberrant disulfide bonds, causes this misfolding [34,55,56]. Studies demonstrate 25–30% of the newly synthesized HLA-B27 heavy chains forming disulfide-linked complexes in the ER [55,56]. No other naturally occurring MHC class I allele has been shown to misfold, although only a few have been studied so far [48]. The B-pocket plays an important role in the misfolding process, as misfolding is corrected by replacing the HLA-B27 B-pocket with one from HLA-A2 [57].

There are three characteristics of protein misfolding: first, there is prolonged binding to the ER chaperone BIP; second, formation of aberrant disulfide bonds with forming of complexes is seen; and finally, heavy chains can be detected undergoing ER-associated degradation (ERAD) [20,48].

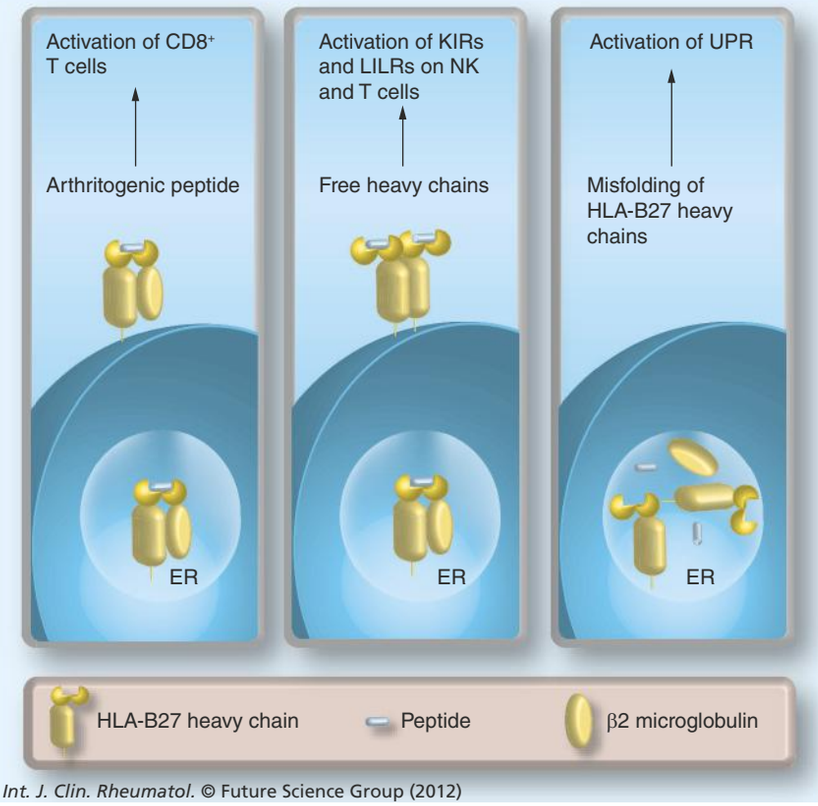

Figure 2. The potential role of HLA-B27 in the pathogenesis of ankylosing spondylitis.
ER: Endoplasmic reticulum; KIR: Killer cell immunoglobulin-like receptor; LILR: Leukocyte immunoglobulin-like receptor; NK: Natural killer; UPR: Unfolded protein response.

In general, the consequences of protein misfolding depend on the nature and severity of the folding defect, the relative importance of the gene product and whether protein quality control processes have intervened sufficiently [32]. Possible downstream events are activation of the ERAD, deposition as an insoluble aggregate in the cell or the extracellular tissue environment (toxic proteins) and activation of a homeostatic response to ER stress, known as the unfolded protein response (UPR) [20,32].

In AS pathogenesis, the current hypothesis postulates that an initial innate immune stimulus activates upregulation of MHC I molecules, which triggers the UPR, by misfolding of HLA-B27, and sensitizes cells to further proinflammatory cytokine production [20].

ER stress & the UPR

Cells have different mechanisms to oppose exogenous sources of stress (e.g., radiation, hypoxia, nutrient deprivation, free radicals, toxins, microbial pathogens)[58]. When unfolded or misfolded proteins accumulate in the cell, ER stress, a form of endogenous cell stress, ensues and the cell activates multiple signaling pathways, together called the UPR [48,58–60]. If successful, the UPR enhances the cell's ability to fold, secrete and degrade proteins. Depending on the severity and duration of the stress and whether cells fail to resolve protein folding defects and restore homeostasis in the ER, the outcome of the UPR can be apoptosis [48].

The UPR was originally described in yeast cells, where there is a single UPR pathway. In higher eukaryotic organisms the UPR is more complex with a three-pronged signal transduction pathway [58]. The UPR is particularly important in cell types that have metabolic and immune functions [60]. Yeast defective in the UPR and ERAD are lethal [59].

Three distinct ER-localized transmembrane proteins play a key role in the UPR: IRE1, PERK and ATF6 [48,58–60]. Those three proteins all have an ER luminal, a transmembrane and a cytosolic domain [58–60]. The ER luminal domain senses misfolded proteins, whereas the cytosolic domain transmits signals to the transcriptional or translational apparatus [60]. The precise activation mechanism of the proximal ER stress sensors is not fully understood. In the absence of ER stress, thus in an inactive state, IRE1, PERK and ATF6 are all sequestered in inactive complexes with BIP (also called GRP78 or HSPA5), an ER-resident protein chaperone [58,59]. The generally accepted hypothesis

postulates that accumulated unfolded proteins bind BIP, which then dissociates from IRE1, PERK and ATF6 and activates the underlying pathways [58]. When BIP dissociates, IRE1 and PERK will oligomerize and trans-phosphorylate other IRE1 and PERK molecules in the complex. ATF, on the other hand, will move to the Golgi complex after dissociation (FIGURE 3) [48,58–60]. Although this hypothesis is commonly supported, it is probably an oversimplification of the complex interactions between diverse signals that are necessary and/or sufficient to activate the UPR [60].

IRE1

IRE1 has two isoforms: *IRE1α*, which is expressed ubiquitously, and *IRE1β*, with an expression limited to gut epithelial cells [58]. Deletion of *IRE1α* in mice leads to an embryonic lethal phenotype [59]. IRE1 has both a kinase and an endoribonuclease function. The downstream consequences of IRE1-mediated kinase activity have yet to be fully elucidated. Activated IRE1 binds TRAF2, which promotes activation of JNK through ASK1, leading to autophagy and apoptosis [58]. The endoribonuclease function of IRE1 is better understood. IRE1 excises a 26-nucleotide sequence from XBP1 mRNA. This splicing causes a translational frame shift with the formation of XBP1s, a XBP1 isoform with potent activity as a transcription factor. XBP1s then translocates to the nucleus and activates specific target genes. On the other hand, the unspliced form of XBP1 (XBP1u) is rapidly degraded [58,60].

PERK

In addition to its oligomerization and trans-phosphorylation, activated PERK will also phosphorylate and inactivate EIF2α, which leads to an arrest of most mRNA translation by inhibition of the assembly of the 80S ribosome [60]. The arrest of translation will cause a decrease of ER stress and, in this way, promotion of cell survival. Moreover, phosphorylated EIF2α will also increase expression levels of *ATF4*, a transcription factor, with transcription of UPR target genes as a result [58].

ATF6

Mammals have two homologous ATF6 proteins, ATF6α and ATF6β. ATF6α participates in the induction of UPR genes, whereas ATF6β seems to have only a minor role in the UPR. When ER stress occurs, ATF6α translocates to the Golgi, where it is cleaved by S1P and S2P. The cytosolic ATF6α fragment (ATF6f) then translocates to the nucleus and activates the transcription of multiple target genes [58,59].

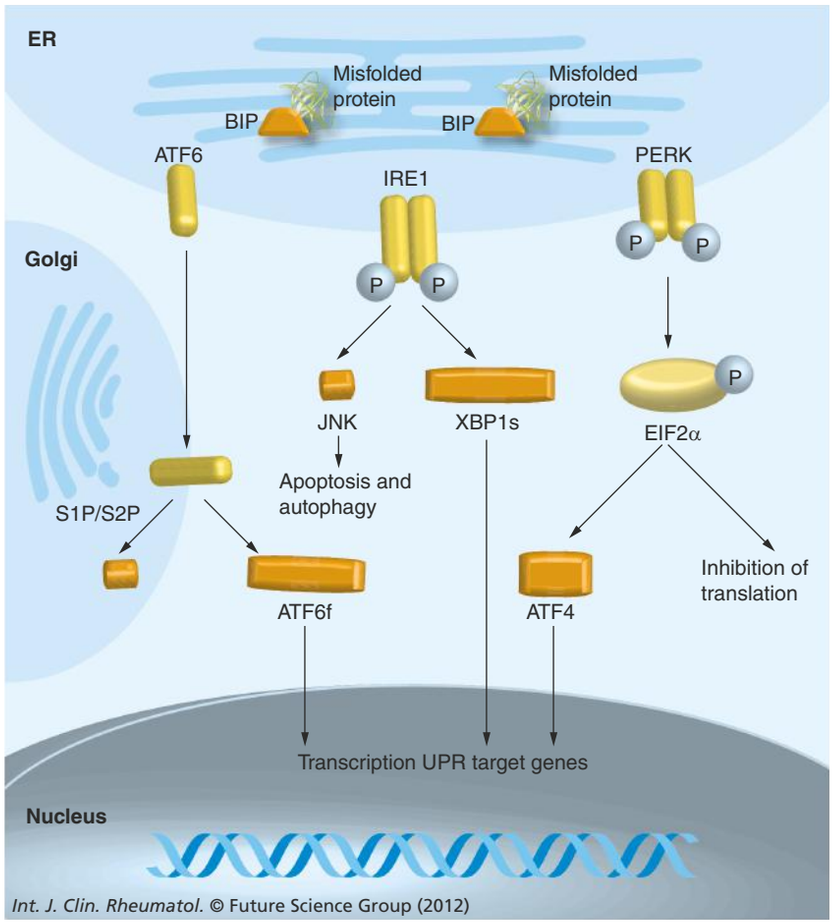

Figure 3. The unfolded protein response is dependent on three different cascades.
ER: Endoplasmic reticulum; UPR: Unfolded protein response.

UPR target genes include: ERAD proteins, such as EDEM; ER-localized chaperones, such as *ERdj4*, *HEDJ* and *DNAJC3*; glycosylation proteins, such as *RAMP4*; and protein disulphide isomerases, such as *PDI-P5* [58]. These chaperones and enzymes promote protein folding, maturation, secretion and ER-associated protein degradation [60]. Other target genes are proteins involved in the UPR pathways, such as *BIP*, *XBP1* and *CHOP*, a pro-apoptotic transcription factor [58]. Deletion of the gene encoding *CHOP* is known to protect the cell against ER-induced cell death, but the mechanism by which CHOP induces apoptosis stays unknown [48].

Activation of UPR in *HLA-B27*-transgenic animals in human AS patients

Some studies with *HLA-B27*-transgenic animals support the UPR hypothesis. Turner *et al.*

showed, for the first time, an activated UPR in bone marrow-derived macrophages from *HLA-B27*/human *β2m* transgenic rats with active disease [61]. Contrarily, there was no activation of the UPR seen in bone-derived macrophages from premorbid animals. Triggering macrophages from these premorbid rats by IFN-γ (as activator of the immune system) led to upregulation of *HLA-B27* expression, with misfolding and activation of the UPR as a result. HLA-B27 heavy-chain misfolding and activation of the UPR was not continuously active in *HLA-B27*-transgenic animals, but could be induced by increased *HLA-B27* expression. As there was no active UPR seen in spleen and thymus cells from these animals (even those with active inflammation), the UPR is also thought not to be a widespread phenomenon in these rats but to be dependent on cell type and exogenous factors [61]. A second study by Turner *et al.* confirmed the temporal and quantitative relationship between *HLA-B27* upregulation and activation of the UPR in macrophages from *HLA-B27*/human *β2m*-transgenic rats [62]. On the other hand, overexpression of additional human β2m in rats with high transgenic copy number of *HLA-B27* heavy chains, by Tran *et al.*, led to absence of colitis but, unexpectedly, to a higher prevalence, severity and duration of arthritis in these animals. It is assumed that additional human β2m leads to stabilization of the HLA-B27 heavy chain with less misfolding and in this way a less-active UPR. The authors conclude that HLA-B27 misfolding may play a role in gut inflammation but not in arthritis and spondylitis in patients with AS [63]. As activation of the UPR was not measured in this study after upregulation of HLA-B27, this conclusion may be premature.

There is some evidence of an active UPR in human AS patients. A few studies demonstrate enhanced expression of *BIP* and other UPR-related genes (e.g., *CHOP* and *XBP1*) in mononuclear cells of peripheral blood samples or synovial fluid from patients with spondyloarthritis in comparison to healthy controls or patients suffering from osteoarthritis [64–66]. A recent study, on the other hand, could not show a significantly increased UPR-regulated gene expression in peripheral blood macrophages from AS patients in comparison to controls (neither at baseline, nor after stimulation with IFN-γ and lipopolysaccharide) [67]. A possible explanation for the difference between those negative data and the positive data in *HLA-B27*-transgenic animal studies from Turner *et al.* may be that the *in vitro* system Zeng *et al.* used is too simplistic compared with the real complex *in vivo* milieu. Another possible explanation may be that the upregulation of the *HLA-B27* molecules was not high enough to trigger the UPR, since *HLA-B27* was only upregulated by twofold in contrast to the four- to ten-fold increase found in the transgenic animals [67].

The UPR & the IL-23/Th17 axis

There is evolving evidence that the UPR is linked to inflammation and joint involvement in AS through the IL-23/Th17 axis. In 1986, the concept of distinct types of Th cells was introduced, which was based on the types of cytokines the T cells produce when they are stimulated to differentiate. The lymphocytes were named Th1 cells, important for clearing intracellular pathogens, and Th2 cells, necessary for clearing extracellular organisms and robust humoral immunity [32,68]. More recently, T cells were shown to produce cytokines that could not be classified in this scheme. One of these cytokines was IL-17, and the cells that produce IL-17 were named Th17 cells [68]. Th17 cells may have evolved as another arm of the adaptive immune response for enhanced protection against extracellular bacteria, protozoa and fungi and can generate an inflammatory response that is dominated by neutrophils [32,68]. Initially, differentiation of Th17 cells appeared to require TGF-β combined with IL-6 and/or IL-1. Now, it has been cogently shown that Th17 cells can differentiate in the absence of TGF-β, using a combination of IL-23 and IL-6 [69]. IL-23 is a heterodimeric cytokine consisting of two subunits, a protein called p19 and the p40 subunit of IL-12 [68], and plays a key role in driving Th17 cells to produce proinflammatory cytokines including IL-17 [70]. The loss of IL-23 in genetically deficient mice made the animals highly resistant to the development of autoimmunity and inflammation [68]. There is evidence in humans that IL-17 is not only produced by T cells, but also by mast cells, neutrophils and innate lymphoid cells [71]. A recent study from Appel *et al.* showed a significant higher number of IL-17-positive cells in the subchondral bone marrow of affected facet joints in AS patients compared with osteoarthritis patients. The clear majority of the IL-17-positive cells were found among the CD15-positive neutrophils and the MPO-positive cells of the myeloid lineage, while CD3-positive T cells and mast cells constituted only a small proportion of IL-17-producing cells [72].

There are some experimental data linking AS, and possibly the UPR, to the *IL-23*/Th17 axis.

The first convincing evidence was the identification of the association between genetic polymorphisms of the IL-23 receptor and AS. The *IL-23* receptor is also known to be associated with inflammatory bowel disease, psoriasis and psoriatic arthritis [28]. Some other evidence is obtained by a study showing that macrophages from *HLA-B27*/human *β2m*-transgenic rats undergoing the UPR (stimulated with pharmacological inducers) are polarized to produce more IL-23 and IFN-β in response to lipopolysaccharide (a Toll-like receptor agonist). At the same time, the rats exhibit Th17-cell expansion and activation in the colon, temporally related to the development of colitis. The authors conclude that HLA-B27 misfolding and the generation of ER stress with activation of the UPR may lead to an augmented Toll-like receptor-mediated induction of IL-23. IL-23 may then sustain Th17 cells and drive the production of IL-17 [70]. An experimental setup with human macrophages from AS patients also showed a significant increase in IL-23 production in response to LPS compared with macrophages from healthy controls. However, in this study, the increase in IL-23 production was not linked to an activated UPR. Even in the absence of an activated UPR, IL-23 production was upregulated [67].

Other recent studies in *HLA-B27*/human *β2m*-transgenic rats show enhanced IL-17 production from $CD4^+$ T cells after stimulation by dysfunctional dendritic cells, as they are seen in *HLA-B27*/human *β2m*-transgenic rats. These results suggest a critical role for the defective costimulatory capacity of dendritic cells in the induction of IL-17-producing T cells [73,74].

IL-17 blockade is promising as a therapy for AS, but larger long-term studies are warranted as only one small clinical study with a lot of drop-outs in the placebo group has been reported [75]. At the same time, further investigations are needed to find out which cells respond to the increased production of IL-23 and which IL-23-induced cytokines apart from IL-17 may contribute to AS [69]. In this light, a key role for IL-23 and IL-22 in AS pathogenesis was recently proposed [76]. An enthesis-associated T-cell population ($CD4^-CD8^-$ $\gamma ROR^+IL\text{-}23R^+$) was identified and responsive to IL-23. These cells appear primed to support both inflammation and new bone formation, the cardinal features of AS. Interestingly, the downstream events after IL-23 and IL-23 receptor interaction do not seem dependent on IL-17. Although exciting and novel, there is a great need to demonstrate the translational relevance of the data, exclusively obtained in mice [76,77].

Biomechanics

Biomechanical factors may play an important role in the pathogenesis of spondyloarthritis. Mechanical forces serve as important regulators at the cell and molecular levels, in an equally strong way as chemical cues do. Mechanical loads will be transmitted across structural elements that are physically interconnected and will be distributed to individual cells through their adhesions to the extracellular matrix support scaffolds. The extracellular matrix also plays an important role in mechanotransduction [78]. One of the best known groups of mechanoreceptors is the group of integrins. Integrins are transmembrane molecules, linking the extracellular matrix with the intracellular cytoskeleton, and are among the first molecules to sense a mechanical stress applied at the cell surface [79]. They transmit this stress signal intracellularly by specific molecular pathways with an important downstream role for transcription factors, such as NF-κB [78]. As NF-κB owns the dual capacity of regulating inflammatory responses on the one hand and linking mechanical stimuli to gene transcription on the other hand, it is thought to play an important role in coupling mechanical factors to inflammation in human diseases [80].

Patients with spondyloarthritis have a propensity for inflammation at the entheses [81]. This process is called enthesitis and is a hallmark of spondyloarthritis, which can distinguish it from other rheumatic diseases [82]. The enthesis is the insertion of a ligament, tendon or joint capsule to the bone [83]. Healthy entheses are avascular, but there appears to be a vascular invasion of enthesis fibrocartilage with aging, as a physiologic response to microdamage [84].

Initially, enthesitis was considered to be a focal process. However, radiological studies, by magnetic resonance and ultrasound imaging, show associated involvement of the adjacent bone marrow and soft tissue. It seems that in spondyloarthritis, inflammatory changes may occur at some distance from the original insertion site. Therefore, the enthesis and the surrounding tissues have been called the 'enthesis organ', with the enthesis organ from the Achilles tendon as an archetype [85]. Because of the close link of the enthesis (avascular tissue) and the synovium (proinflammatory and vascular tissue) in an anatomical, functional and physiological way, those two structures can be seen as forming a 'synovio–entheseal complex' [84,86]. In this concept, synovitis in spondyloarthritis may be an anticipated consequence of primary enthesitis [84,85]. The primary enthesitis may be caused by

biomechanical stress as the enthesis is a site of repetitive biomechanical forces, generated during normal movements of the muscles, tendons, ligaments and joints. As the synovium is less subject to biomechanical stress than the enthesis is, the enthesis may be more prone to injury than the synovium [83]. There is not a lot of data, but it seems that biomechanical stress in cell cultures causes a marked change in gene expression, with an estimated 600 stress-responsive genes [80]. Beside this direct effect on gene expression, sustained biomechanical stress will also cause tissue microtrauma, leading to a healing and inflammatory response, mediated by cytokines including IL-1, TNF-α, TGF-β and IL-8 [80]. Microtrauma is not only seen in enthesitis in spondyloarthritis, but is also a feature of other enthesopathies, such as medial and lateral epicondylitis, and Osgood-Schlatter disease [81]. In this way, biomechanical stress may also have an indirect effect on cytokine and transcription factor expression [80].

A few years ago, McGonagle *et al.* proposed an enthesitis based-model for the pathogenesis of spondyloarthritis, where interaction between joint-specific biomechanical stress factors and the innate immune response (e.g., as a reaction on bacterial products) leads to the specific disease localization [80,84,86]. As not every musculoskeletal site suffering biomechanical stress is associated with spondyloarthritis, other factors may also be important for disease localization. One of these factors may be the type of stress, where entheseal insertions undergo compressive and shear stress that may differ from stress at other sites [80]. Another factor possibly playing a role, not in the localization of disease, but in the gravity of inflammation, is HLA-B27, as there is a small study showing a correlation between the extent of bone pathology and HLA-B27 in patients with enthesitis at the plantar fascia, but no correlation between bone edema and HLA-B27 *per se* [87].

The hypothesis that primary entheseal abnormalities trigger secondary synovitis is virtually impossible to study in humans, but can be carried out in animal models. One of these models is the spontaneous arthritis model in DBA/1 mice, characterized by oligoarthritis of the hind paws and more rarely the ankles [84]. Histomorphologic analyses from affected joints demonstrate that ankylosis in this animal model is a result of enthesitis and that synovitis is not necessary for the appearance of clinical arthritis in these mice [88]. In a recent study in *HLA-B27*/human *β2m*-transgenic rats, on the other hand, no single site of enthesitis was seen in the 27 axial and 24 peripheral joints they explored. The authors conclude that the inflammatory process in *HLA-B27*/human *β2m*-transgenic rats primarily affects the stromal tissue and not the insertion of tendons and ligaments, neither in axial nor in peripheral joints [89]. However, some caution is warranted in the interpretation of the histological lesions. The enthesis is resistant to cell invasion. Therefore, inflammatory infiltrates are found in immune privileged sites in close proximity to the actual enthesis, such as the synovium and the bone marrow. This anatomical, cellular and molecular field of interaction was previously defined as the synovio–entheseal complex [84] and may also be applied to some of the images presented from *HLA-B27*-transgenic rats [89]. In addition, the identification of an IL-23-responsive T-cell population within the enthesis of mice would support enthesis-centered concepts [76].

The enthesis is not only prone to inflammation in human spondyloarthritis, but is also a common site for bony spur formation [81]. Ossification at most entheses is commonly endochondral but is occasionally also reported as intramembraneous. There is some evidence that the trigger for this bone formation is mechanical and triggered by teamwork of bone morphogenetic proteins, activins and transforming growth factor. Despite the known role of mechanical stress in new bone formation at the enthesis, it remains unclear if the bone formation leading to ankylosis in spondyloarthritis can occur in the absence of proceeding inflammation [81].

Some evidence links biomechanical strain with ER stress. Application of high-amplitude tensile forces through collagen-coated beads in fibroblasts, which is a model for forces that are delivered to connective tissue cells *in vivo*, is shown to lead to an increased phosphorylation of EIF2α [90]. In a second study, tensional forces applied in fibroblasts led to apoptosis in a PERK-depending way. As neither phosphorylation of IRE1α nor expression of CHOP was essential for force-induced apoptosis in this study, it is thought to be a noncanonical UPR pathway that is induced by tensile forces [91]. With these results in mind, it is possible that the effect of misfolded HLA-B27 heavy chains, with an activation of the UPR, can be exacerbated by mechanical factors.

Conclusion & future perspective

As the real association between *HLA-B27* and AS is still unknown, future studies should keep

their focus on this relationship. First, a well-designed study about activation of the UPR in human AS synovium samples is needed. Another question to be solved in the near future is what specific role IL-23 and Th17 cells play in the pathogenesis of AS. Further investigations are needed to find out if cells other than Th17 cells respond to the increased production of IL-23 and which IL-23-induced cytokines apart from IL-17 may contribute to AS. Finally, as strong experimental evidence about the biomechanical stress hypothesis is lacking, animal studies must be set up to confirm the currently postulated mechanisms. As only the further unraveling of the pathogenesis of AS can lead to the development of new therapeutic agents for AS, intense investigations into the pathogenesis remain of major importance.

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Executive summary

Current concepts of ankylosing spondylitis

- There are many hypotheses with regard to the pathogenesis of ankylosing spondylitis (AS), as the actual cause is not yet known.
- AS is a multifactorial disease and both genetic and environmental factors may play a role in the pathogenesis.

***The potential effect of* HLA-B27**

- The association of AS with *HLA-B27* is among the strongest genetic associations with any common disease, as over 90% of AS patients are HLA-B27 positive.
- Three different hypotheses are proposed that link *HLA-B27* to AS:
  - The arthritogenic peptide hypothesis;
  - The free heavy-chain hypothesis;
  - Endoplasmic reticulum stress/unfolded protein response (UPR) hypothesis.

Endoplasmic reticulum stress & the UPR

- Activation of the UPR is seen in animal models of AS, but strong evidence in human AS patients is still lacking.

The UPR & the IL-23/Th17 axis

- Experimental data show that the IL-23/Th17 axis plays a role in the pathogenesis of AS.
- The UPR may be the link connecting the IL-23/Th17 axis to the development of AS, but evidence is lacking.

Biomechanics

- An enthesitis-based model for the pathogenesis of AS was proposed by McGonagle *et al.*, where interaction between joint-specific biomechanical stress factors and the innate immune response leads to disease localization. Strong experimental evidence is lacking.

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