

Review

Understanding the pathogenesis of spondyloarthritis

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Abstract:

Spondyloarthritis comprises of a group of inflammatory diseases of the joints and spine with various clinical manifestations. The group includes ankylosing spondylitis, reactive arthritis, psoriatic arthritis, arthritis associated with inflammatory bowel disease, and undifferentiated spondyloarthritis. The exact etiology and pathogenesis of spondyloarthritis are still unknown, but five hypotheses explaining the pathogenesis exist. These hypotheses suggest that spondyloarthritis is caused by arthritogenic peptides, an unfolded protein response, HLA-B27 homodimer formation, malfunctioning endoplasmic reticulum aminopeptidases, and, last but not least, gut inflammation and dysbiosis. Here we discuss the five hypotheses and the evidence supporting each. In all of these hypotheses, HLA-B27 plays a central role. It is likely that a combination of these hypotheses, with HLA-B27 taking center stage, will eventually explain the development of spondyloarthritis in predisposed individuals.

Keywords: spondyloarthritis, HLA-B27, pathogenesis, inflammation, arthritogenic peptides, unfolded protein response, ERAP1, gut dysbiosis

1. Introduction

Spondyloarthritis (SpA, also called spondyloarthropathy) is a group of inflammatory diseases of the joints and spine with various clinical manifestations. Five major subtypes of SpA are recognized on the basis of the classification criteria proposed by the European Spondyloarthropathy Study Group (ESSG) [1]. These subtypes include ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis (PsA), arthritis associated with inflammatory bowel disease (SpA-IBD), and undifferentiated spondyloarthritis (uSpA) [2,3]. More recently, a classification into axial and peripheral diseases has been proposed. The main clinical manifestations of SpA are inflammatory pain and stiffness and swelling of the joints and spine. In addition, peripheral arthritis, enthesitis, dactylitis, sacroiliitis, and chronic inflammatory bowel disease (IBD) are often observed [3,4]. Less common symptoms are inflammation of the eye or GI tract, and hearing loss [5].

The exact etiology and pathogenesis of spondyloarthritis are still unknown. However, several lines of evidence indicate that genetics plays an important role in individuals' susceptibility, while environmental factors -resulting in infections and gut dysbiosis- also contribute to SpA pathogenesis. Various studies demonstrated that microorganisms play a role in triggering the disease. Genetically, SpA is strongly associated with the major histocompatibility complex (MHC) class I antigen, HLA-B27. For example, more than 95% of patients with AS possess HLA-B27, whereas its frequency among the general population is less than 10%. The risk of developing AS is as high as 5-7% in HLA-B27-positive individuals [5]. HLA-B27 consists of an alpha chain encoded in the B locus of the MHC region on chromosome 6 and a non-MHC encoded beta chain, β_2 microglobulin (Figure 1). The primary function of HLA-B27 in complex with β_2 -microglobulin is to present short antigenic peptides for recognition to cytotoxic T lymphocytes (CTLs; Figure 1). The ability of HLA-B27 to bind a unique set of peptides may be the underlying reason for its disease association with SpA [6,7]. Three unique features of HLA-B27, including peptide binding specificity, a tendency

to misfold, and the predisposition for forming heavy chain homodimers may contribute to disease pathogenesis [8]. Each of these features has been suggested independently to play a role in the pathogenesis of SpA.

Moreover, HLA B-27 is a highly polymorphic molecule of which currently (April 2020), more than 223 subtypes are known (<https://www.ebi.ac.uk/ipd/imgt/hla/allele.html>). The high number of polymorphisms, in combination with heterozygosity, give a selective advantage to the immune system against the diversity of microorganisms and their antigens. However, extreme polymorphism and potential mutations in the major histocompatibility complex (MHC) increase the chance of developing autoimmune diseases [9]. Thus, these molecules play an essential role in the regulation of the host's immune response by initiating tolerance and CTL or helper T cell responses by presenting peptides to T-cell receptors [10,11]. Among the HLA-B27 alleles, HLA-B*2705 and HLA-B*2704 are the most strongly associated with SpA, whereas, HLA-B*2706 and B*2709 are weakly associated [12]. It is well known that the prevalence of SpA varies strongly between ethnic groups and populations, this can partly be explained by differences in the prevalence of HLA-B27 [3,13]. The prevalence of AS varies from 0.2% to 0.7% among European populations while higher frequencies of AS have been reported in populations from Siberia, Alaska, and Scandinavia [13].

The interaction between bacteria and HLA-B27 alleles plays an important role in the hypotheses of SpA pathogenesis [14]. The evidence that ReA is triggered by *Chlamydia trachomatis* (genitourinary infections) or by other gram-negative enterobacteria, such as *Shigella*, *Salmonella*, *Yersinia*, and *Campylobacter* species provides a solid background for these hypotheses [15]. Also, inflammatory arthritis frequently develops following infection with enteric organisms such as *Clostridium difficile*, *Brucella*, and *Giardia*. The identification of microbial antigens in the synovium of ReA indicates that those antigens can be crucial for ongoing joint inflammation [16]. The fact that some SpA types can be triggered by bacterial infections proves the importance of these organisms in SpA pathogenesis. Therefore, inappropriate immune responses to commensal

bacteria or alterations in gut microbiota were also proposed to be factors in SpA development.

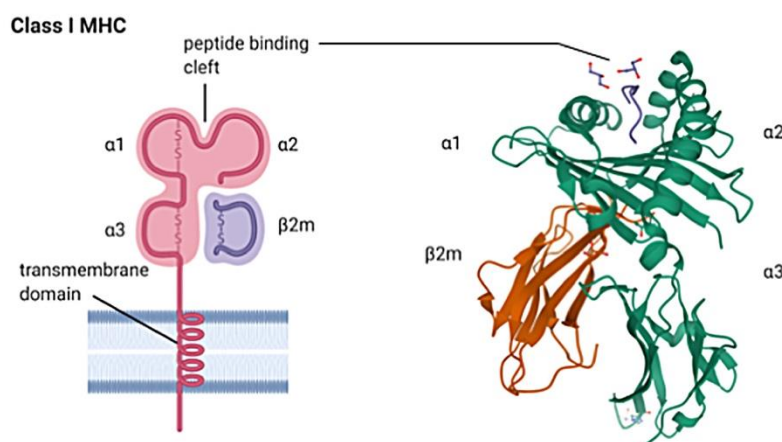


Figure 1. A model depicting the MHC Class I HLA-B27 heavy chain in complex with beta 2-microglobulin ($\beta 2m$). The alpha 1 ($\alpha 1$), 2 ($\alpha 2$), and 3 ($\alpha 3$) domains of the HLA-B27 molecule are indicated on the left. The transmembrane domain inserted into the lipid bilayer of the plasma membrane and the peptide binding cleft composed of $\alpha 1$ and $\alpha 2$ domains of HLA-B27 are indicated by solid black lines. A ribbon model of the HLA-B27 heavy chain structure (green) with bound peptide and in complex with beta 2-microglobulin ($\beta 2m$; orange) is shown on the right. The alpha 1 ($\alpha 1$), 2 ($\alpha 2$), and 3 ($\alpha 3$) domains of the HLA-B27 molecule are indicated. The peptide binding cleft composed of $\alpha 1$ and $\alpha 2$ domains of HLA-B27 is indicated. The bound peptide is shown. Orientation: cell surface at top of picture.

2. Models of SpA pathogenesis: hypotheses

The exact pathogenetic role of HLA-B27 is unknown [17]. There are, however, some predominant theories explaining the role of the HLA-B27 allele in SpA and disease pathogenesis, including the arthritogenic peptide hypothesis, misfolding protein hypothesis, as well as the hypothesis of the cell-surface HLA-B27 homodimer [18].

Numerous hypotheses have been suggested regarding the role of HLA-B27 in the pathogenesis of AS. These are mainly based on rodent animal models. One of the

oldest and major theories for explaining HLA-B27 pathogenicity, is the so-called “arthritogenic peptide” hypothesis. This hypothesis proposes that the presentation of unique sets of antigenic peptides, either self- or bacteria-derived, by HLA-B27 alleles to CD8 T cells, results in cross-reactivity and an HLA-B27–restricted cytotoxic T-cell response. This response subsequently triggers a harmful CD8+ T cell-mediated response in joints and other affected tissues [13,19-22]. The second hypothesis, the “unfolded protein response,” suggests that HLA-B27 tends to fold slowly and often misfolds. The misfolded HLA-B27 molecules accumulate in the endoplasmic reticulum (ER), where they induce a stress response that leads to an unfolded protein response (UPR) and autophagy [20-22]. The upregulation of UPR genes subsequently activates the production of pro-inflammatory cytokines such as IL-17, IL-23, and IFN- γ [21]. Another hypothesis that explains the role of HLA-B27 in SpA pathogenesis is the “formation of homodimers” hypothesis. According to this hypothesis, HLA-B27 heavy chains homodimerize at the cell surface, where they act as pro-inflammatory ligands for humoral or cell-mediated autoimmune responses [12,20]. These homodimers are capable of binding to certain killer cell immunoglobulin-like receptors (KIRs), which are expressed on natural killer cells and T cells, and this leads to the upregulation of the pro-inflammatory cytokine IL-17 [22].

The gene with the second strongest SpA association, Endoplasmic Reticulum Aminopeptidase 1 (*ERAP1*), trims peptides to optimal sizes for antigen presentation. Loss-of-function alleles of *ERAP1* lead to abnormal HLA-B27 presentation and accumulation of intracellular free heavy chains, which can result in changes in peptide processing with pathological consequences [23,24]. Non-genetic contributors to SpA are certain infections and gut dysbiosis. Many studies indicate a link between intestinal bacterial composition, in particular gut dysbiosis, and SpA. In this review, we will discuss the hypotheses to explain the disease mechanisms of SpA pathogenesis. We will also propose a hypothesis that combines the previously proposed hypotheses.

2.1. Arthritogenic peptide hypothesis

The arthritogenic peptide hypothesis is one of the oldest hypotheses explaining the association between HLA-B27 and SpA [25]. It proposes that some HLA-B27 subtype alleles, due to their characteristic amino acid residues, bind a specific arthritogenic peptide that is recognized by CD8+ T cells [26]. This hypothesis assumes that the natural ligands of HLA-B27 are target antigens of autoimmune T cells induced by cross-reactive bacterial peptides. Peptide presentation by HLA-B27 is affected by subtype polymorphisms that affect the peptide-binding groove [27]. Consequently, a set of unique antigenic peptide epitopes with key conserved anchor residues are recognized by specific HLA-B27 alleles. The arthritogenic peptide hypothesis is based on the peptide binding specificity of HLA-B27, the concept of molecular mimicry, and the idea that activation of T cell responses by an external antigen may result in autoimmunity. The initial pathogenic event in SpA could be the activation of HLA-B27 by CTL responses against bacterial peptides. Cross-reaction of some activated CTL with a self-peptide would subsequently lead to autoimmune tissue damage and inflammation [28].

Various studies support the arthritogenic peptide hypothesis. For instance, HLA-B27-restricted CD8 T cell responses specific for *Salmonella* or *Chlamydia* have been identified in patients who developed reactive arthritis following infections with these pathogens [29,30]. Also, B27- restricted CD8+ CTL were found in the synovial fluid of ReA patients [31,32]. A set of peptides derived from enteric organisms were identified that bound to and have sequence homology with, HLA-B27 [33]. AS patients also have a higher probability of having autoantigenic antibodies directed against *Klebsiella* that are cross-reactive against B27 in comparison to HLA-B27 positive but healthy individuals [34]. Moreover, investigation of the HLA-B27 peptidome identified more than a thousand peptides from HLA-B27+ cell lines, half of which were found to tightly bind to HLA-B27 alleles. Twenty eight of these peptides were considered to be arthritogenic peptides as they were derived from cartilage or bone proteins that had

high homology with proteins from enteric bacteria. These findings suggest that these antigenic peptides can induce a T-cell mediated immune response that results in SpA pathogenesis [25,35]. Along with bacterial peptides, viral peptides could also contribute to SpA pathogenesis. In some SpA patients, cross-reactive CD8 T cell responses that recognized both an epitope from Epstein-Barr virus (EBV) and a self-peptide derived from the vasoactive intestinal protein receptor (VIPR) were identified [36]. However, EBV infection is not recognized as a SpA trigger and these findings warrant further investigation. Major support for the arthritogenic peptide hypothesis comes from studies of HLA-B27 alleles and their differential association with SpA pathogenesis. For example, some alleles such as HLA-B27*05, HLA-B27*02, and HLA-B27*04 are strongly associated with the disease, whereas some other alleles (HLA-B27*06 and HLA-B27*09) were not or only weakly associated with disease pathogenesis [37].

Nevertheless, conclusive evidence that directly implicates this mechanism in the pathogenesis to support this arthritogenic peptide hypothesis is still lacking [20]. Recently, HLA-B27 peptidomes from SpA-related and unrelated alleles were studied, and no clear distinction in qualitative differences between these groups was found, indicating that binding preferences of HLA-B27 alleles do not solely explain disease association [38,39].

2.2. The unfolded protein response hypothesis

The unfolded protein response (UPR) hypothesis is another theory explaining the role of HLA-B27 in SpA pathogenesis. Compared with other MHC molecules, HLA-B27 tends to slowly fold or even misfold in the endoplasmic reticulum (ER), and to accumulate in the ER for extended periods of time, which may have implications for pathogenesis [40]. The accumulation of misfolded forms of HLA-B27 in the ER leads to ER stress that induces the UPR, which is a homeostatic mechanism that aims to return the cell to its normal state [41].

UPR suppresses protein translation and upregulates ER chaperone molecules such as immunoglobulin heavy-chain-binding protein (BiP) and endoplasmic reticulum-localized DnaJ 4 (ERdj4). It also activates transcription factors such as CCAAT-enhancer-binding protein homologous protein (CHOP) and increases the production of pro-inflammatory cytokines (IL-23, INF- β , and IL-1) [42]. Indeed, UPR affects cytokine production on multiple levels, from stimulation of surface receptors to activation of inflammatory signaling pathways, in particular the IL-23/IL-17 signaling pathway [43]. Autophagy induced by ER stress and UPR also increase the production of IL-23 [44].

Misfolded HLA-B27 molecules in the ER are disposed of by ER-associated degradation (ERAD), therefore, slow folding or accumulation of misfolded HLA-B27 increases levels of ERAD and stimulates UPR and autophagy, in particular during inflammation when HLA-B27 production is high [44,45].

The slow-folding kinetics and partial misfolding of HLA-B*2705 lead to the activation of NF- κ B, which leads to increased production of pro-inflammatory cytokines, such as TNF, IL-1, and IL-6. This hypothesis is concordant with observations in transgenic mice showing that the absence of β 2 microglobulin is required for the development of arthritis [46,47].

In support of the UPR hypothesis, it was shown that misfolding and UPR occur in HLA-B27 transgenic rat models of AS and in the gut and synovial tissues of SpA patients [48]. Bone marrow-derived macrophages from HLA-B27 transgenic rats show evidence of HLA-B27 misfolding after cytokine stimulation and this was associated with IL-23 production [49,50]. These HLA-B27 transgenic rats have functional alterations in the number of cell populations, which in turn can be correlating with misfolding. Moreover, the introduction of additional copies of the human β 2-microglobulin gene reduced B27 misfolding increases the incidence and severity of arthritis [51]. Nevertheless, studies of tissues of AS patients did not suggest that UPR and ER stress were associated with inflammation and HLA-B27 misfolding [52,53].

Therefore, HLA-B27 was found to lead to misfolding and ER stress in both *in vitro* and animal studies; however, currently, there is no evidence of involvement of this process in SpA pathogenesis in humans.

It was demonstrated that in M-CSF-derived macrophages from AS patients, UPR pathway genes (CHOP and BiP) and IL-23 are upregulated in comparison to healthy controls [54]. Recently, it was suggested that autophagy but not UPR is responsible for the regulation of IL-23 expression in the gut of AS patients [53]. This hypothesis is further supported by the observation that disease severity is unaffected by the lack of functional CD8⁺ T cells but requires CD4⁺ T cells in HLA-B27 transgenic rats [37,55].

HLA-B27 misfolding and UPR cellular environments are associated with enhanced Salmonella replication. It was demonstrated that the misfolding of HLA-B27 occurred without significant UPR activation in the gut of AS patients [56]; nevertheless, elevated BiP expression and UPR activation were observed in synovial fluid and cytokine-induced macrophages of AS patients [57,58].

2.3. HLA-B27 homodimer formation hypothesis

The ability of HLA-B27 to form disulfide-bonded homodimers is one of the unique characteristics of this molecule. These homodimers are expressed on the cell surface of a variety of cell types, including natural killer cells (NKs), CTLs, and B lymphocytes [12]. It has been suggested that homodimer formation is a sign of HLA-B27 misfolding within the ER and that the accumulation of misfolded protein potentially results in a pro-inflammatory stress response [40]. HLA-B27 homodimers can facilitate the production of Th17 cells and can also upregulate the expression of IL-23/IL-1 in the Th17 cells [41]. HLA-B27 homodimers can bind KIRs and leucocyte immunoglobulin-like receptors (LILRs), which are expressed on NKs and T cells, and thus upregulate the IL-23/IL-17 signaling pathway and inflammation [22,59].

The ER protein misfolding has a variety of biological effects depending on the nature

and quantity of the misfolded proteins, as well as on the severity of the defect. UPR activation can alter cytokine production, including increased production of pro-inflammatory cytokines IL-27, which is essential for SpA pathogenesis. Altered production of TNF and IFN- γ may play a crucial downstream role that contributes to the unique spondyloarthritis phenotype [8].

There is much evidence supporting the HLA-B27 homodimer hypothesis. Both KIRs and LILRs play crucial roles in immune responses, including differentiation of macrophages and dendritic cells, T-cell survival, and activation of regulatory T cells (Tregs) [60]. The KIR3DL2 receptor was shown to recognize HLA-B27 homodimers with higher affinity than normal HLA-B27 heterodimers [18]. So far, seventeen KIR genes have been identified, and interaction of KIR3DL2 with HLA-B27 free heavy chains was shown to have pro-inflammatory effects in NK and T cells, and was linked to elevated Th17 expression in AS patients [59,61,62]. The binding of KIR3DL2 with HLA-B27 homodimers was also observed to facilitate the survival and differentiation of CD4⁺ T cells in SpA patients. In addition, it increased production of pro-inflammatory cytokines such as IL-17, TNF, and IFN- γ [18,60,63]. Moreover, in HLA-B27 positive SpA patients, KIR3DL2⁺ NK cells and CD4⁺ T cells were increased in peripheral blood and showed a higher level of cytotoxicity and IL-17 production than in HLA-B27 negative SpA patients [57,64]. Thus, the binding of HLA-B27 homodimers to KIR and LILR facilitates inflammation by enhancing the survival of NK and T cells and affecting the differentiation of LILR-expressing antigen-presenting cells [13,37,60]. In addition, the arthritis-associated HLA-B*27:05 allele was shown to form more cell-surface homodimers than the non-associated HLA-B*27:09 allele [65].

Nevertheless, some research suggests that HLA-B27 homodimers are not very important in SpA pathogenesis. The ability to form homodimers of eight common HLA-B27 alleles (HLA-B*27:02 to HLA-B*27:09) was analysed and no major differences were found between disease-associated and non-associated HLA-B27 allotypes. In particular, HLA-B*27:03, an arthritis-associated HLA-B27 subtype, has a

reduced ability to form cell-surface homodimers. These findings challenge the role of HLA-B27 homodimer formation in SpA pathogenesis and suggest that other features of HLA-B27 play a more central role in disease mechanism [66].

2.4. *The hypothesis of malfunctioning ERAPs*

The genetic association of polymorphisms in the ER aminopeptidases, ERAP1 and ERAP2, with AS is the second strongest after HLA-B27 and contributes approximately 15-25% of the population risk [23,67]. This association was first reported in the UK and the USA and was validated in independent studies with other populations [21,68-70]. Together, HLA-B27 and ERAP explain 70% of the genetic risk of developing SpA [71,72]. Endogenous proteins presented to MHC I class molecule are transported to the ER and trimmed to a suitable length for MHC-I binding by ERAP1 and ERAP2 [73]. ERAP-1 is a non-MHC SpA associated gene and member of the zinc metallopeptidases, which is involved in antigen cross-presentation and regulates the shedding of tumor necrosis factor receptor 1 (TNFR1) [74].

ERAP1 has two major functions. The first is trimming of processed N-termini of peptides in the ER to a suitable length (8-10-mer) to allow binding to MHC class I molecules and presenting them on the cell surface of T cells. A failure in this function of ERAP1 causes changes in the normal expression of HLA-B27 and increases intracellular free heavy chain forms of HLA-B27 in antigen-presenting cells [39,75]. The second function of ERAP1 is the cleavage of cytokine receptors that are expressed on the cell surface, such as TNFR1, IL6R2, and IL1R2, which leads to a reduction of pro-inflammatory signaling [68,76]. These cytokine receptors play, however, no major role in SpA development [69,77].

The fact that the strong genetic association of AS with *ERAP1* was only found in HLA-B27 positive individuals indicates that ERAP1 most likely interacts with HLA-B27 within the antigen-processing and presentation pathway [36,67]. So, abnormal peptide

processing and antigen presentation is a crucial contributor to SpA pathogenesis [69,78]. Epistatic interactions with ERAP1 were also identified for Behçet's disease with HLA-B51 [79], and for psoriasis with HLA-Cw3 [80]. These interactions suggest the possibility of similar HLA-driven pathogenic mechanisms in these diseases. Moreover, it was shown that *ERAP1* polymorphisms can also affect the accumulation of HLA-B27 homodimers on the cell surface [37,61].

The exact mechanism behind the genetic association with *ERAP1* is unclear. Nevertheless, it was found that various *ERAP1* polymorphisms contribute to the altered disease risk. For example, rs30187, a polymorphism that changes the amino arginine to the more enzymatically active lysine at position 528 (R528L), increases disease risk [81]. In addition, loss-of-function *ERAP1* polymorphisms affect HLA-B27 heavy chain dimerization and misfolding [69,81]. Moreover, AS patients appear to have higher expression of the *ERAP1* gene (Campbell et.al 2011), so overexpression of *ERAP1* may be one of the mechanisms explaining SpA pathogenesis. More importantly, it was demonstrated that the loss of ERAP1 function in humans and mouse models leads to changes in the MHC I peptidome, indicating it is important for shaping the MHC I peptide repertoire [82]

ERAP1 polymorphisms may play a key role in SpA pathogenesis through variation in the peptide repertoire bound to HLA-B27, and through the generation of abnormal intracellular and extracellular forms of HLA-B27 by affecting either ER misfolding or the export of pro-inflammatory B27 forms [83]. Various *ERAP1* polymorphisms have been identified that are associated with the disease [84]. For example, the *ERAP1* polymorphisms rs30187, rs27044, and rs2287987 were associated with AS susceptibility in a Polish population [85]. In an Iranian population, the *ERAP1* polymorphisms rs30187 and rs2287987 were associated with the risk of developing AS [24]. Interestingly, the polymorphism rs75862629 provided protection from AS in HLA-B27 positive individuals in Sardinia by regulating the expression of both ERAP1 and ERAP2 [86]. However, the effect of *ERAP1* polymorphisms on the susceptibility to SpA

may vary among ethnic groups; therefore, identification of genetic variations in different populations could be important for understanding SpA pathogenesis [24,84].

The association between ERAP1 polymorphisms and the development of AS also supports the arthritogenic peptide hypothesis. The function of ERAAP (the ERAP1 equivalent in mice) is, in mice, important for MHC class I peptide processing and presentation, and its destruction leads to an abnormal CD8 T cell response [87]. Moreover, *ERAP1* polymorphisms might be associated with SpA by increasing TNF receptor shedding [76], there were, however, significant differences in mRNA expression of certain cytokines between AS patients with different *ERAP1* haplotypes [24,88].

In addition to *ERAP1* polymorphisms, *ERAP2* polymorphisms may also be associated with AS, despite that ERAP2 is not involved in receptor shedding [25]. In addition, *ERAP1* and *ERAP2* polymorphisms are associated with a variety of inflammatory diseases, including AS. The relationship between ERAP1 and HLA-B27 might be crucial for SpA development, as it was shown that the inhibition of ERAP1 leads to changes in cells with disease-associated HLA-B*27:04 or HLA-B*27:05, but not in cells with the non-associated HLA-B*27:06 or HLA-B*27:09 [21].

In summary, some longer peptides that are generated by abnormal peptide processing due to defective ERAP1 play a crucial role in the pathogenesis of SpA by changing the antigen presentation pathway, resulting in activation of an inflammatory and pathogenic immune response [24,89].

2.5 Gut inflammation and dysbiosis hypothesis

The most recent hypothesis explaining SpA pathogenesis is the role of HLA-B27 in shaping the gut microbiome, and HLA-B27-mediated changes in the gut microbiome associated with disease susceptibility [39]. The gut microbiome is a complex homeostatic ecosystem consisting of trillions of bacteria, and plays important roles in

the development of the host's immune system, in food digestion, and the intestinal epithelial barrier [39,90]. Changes in gut microbiome composition are correlated with autoimmune diseases through mechanisms such as the activation of the immune response, molecular mimicry, and increased intestinal permeability [39].

HLA-B27 could influence SpA pathogenesis by shaping the gut microbiome. There are multiple lines of evidence supporting this theory. It was found that HLA-B27 facilitated the survival of Gram-negative intracellular bacteria in HLA-B27 expressing HeLa cell lines [91,92]. There is a known correlation between host genetic background and changes in the gut microbiome that establish an inflammatory state which can promote arthritis [44,93]. The gut microbiome in HLA-B27 transgenic rats is significantly different from the gut microbiome in littermate controls [21,94]. The relationship between the gut microbiome and arthritis has been actively investigated in human studies [44].

Moreover, it was hypothesized that HLA-B27-induced changes in the gut microbiome can be an important step in the development of SpA [90,95]. It was shown that gut dysbiosis and inflammation could facilitate innate immunity activation and Th17 cell expansion in HLA-B27 transgenic rats [96]. A lot of evidence supports the relationship between gut inflammation and SpA. For example, 7 % of AS patients also have IBD. Conversely, 10-50 % of IBD patients develop SpA [90]. Therefore, it is crucial to understand how HLA-B27 shapes the gut microbiome and impacts SpA development.

The advent of genomic technologies and advancement in bioinformatic analyses will facilitate the comprehensive understanding of gut microbiome changes of SpA patients and have already facilitated significant improvements in this field [39]. Various studies have shown that the presence of specific changes in the gut microbiome can be responsible for the initiation of disease, such as an increased number of opportunistic pathogens, gut dysbiosis, and a shift in the composition of commensal bacteria [97,98]. Gut dysbiosis could trigger antigenic activation of pathogenic effector cells of the immune system, which in turn facilitate chronic

inflammation. Increased gut permeability is a characteristic and pathogenic pathway of SpA development. Tight junctions between intestinal epithelial cells are dysregulated, which leads to increased gut permeability and harms mucosal immunity by altering normal regulation of gut microbiota and secretion of pro-inflammatory cytokines [99]. The evidence for this hypothesis is that intestinal permeability is increased in AS patients and that bacterial by-products have been detected in the synovial fluid of joints of AS patients [100].

Through various metagenomics studies, the gut microbiome composition of SpA patients was demonstrated to be different from healthy controls. For example, a higher number of *Lachnospiraceae*, *Veillonellaceae*, *Prevotellaceae*, *Porphyromonadaceae*, and *Bacteroidaceae* was observed in patients with AS [101,102]. A metagenomics study analysed the gut microbiome composition in three cohorts of volunteers: SpA patients, RA patients, and healthy controls. A disease-specific dysbiosis was identified in SpA and RA patients, but not in healthy controls; in particular, a significantly increased amount of *Ruminococcus gnavus* were found in SpA patients, and its levels were correlated with IBD severity in this patient cohort. Moreover, significantly different gut microbiome compositions were detected in healthy HLA-B27 positive and negative individuals, which suggests that genetic background might affect gut microbiota [103]. Deep shotgun sequencing of 211 Chinese individuals identified 23,709 genes and 12 metagenomic species that were differentially expressed between AS patients and healthy controls. *Prevotella melaninogenica*, *Prevotella copri* and *Prevotella* sp. were increased while *Bacteroides* spp. were decreased in AS patients [98,104].

There is substantial evidence suggesting that the interaction between host genetics and the gut microbiome is a key driver of the pathogenesis of SpA [105]. Pathogenic bacteria and possibly viruses occurring in the gut of SpA patients contribute to dysbiosis and lead to immune dysfunction and modulation of the innate and adaptive immune responses [98].

3. Conclusions

Despite intensive research, the exact mechanism of SpA pathogenesis and the pathogenic role of HLA-B27 remain unclear. Five major hypotheses each explain the role of HLA-B27 in SpA: the arthritogenic peptide hypothesis, the misfolded HLA-B27 hypothesis, the cell-surface homodimer formation hypothesis, the hypothesis on malfunctioning of ERAPs, and the hypothesis based on gut microbiome changes. In the previous sections, these hypotheses were described, and it was shown that there is ample evidence to support each of these. It is possible that all or most of these mechanisms contribute to disease in individuals. Moreover, taking into account that the frequencies of HLA-B27 alleles and *ERAP1* polymorphisms are ethnic-specific, it is important to understand that SpA pathogenesis could well be the result of different combinations of these mechanisms in different populations (Figure 2).

The link between these hypotheses could be canonical and non-canonical features of HLA-B27 and *ERAP1* polymorphisms. In particular, *ERAP1* polymorphisms may contribute to all three models (arthritogenic peptide theory, unfolded protein response, and homodimer formation) that explain how HLA-B27 causes AS. *ERAP1* could contribute to SpA by altering antigen presentation due to abnormal aminopeptidase activity and dysregulation of peptide processing. Altered rates of peptide trimming mediated by *ERAP1* could lead to cell surface presentation of aberrant peptides for the HLA-B27 ligand. This facilitates modulation of the immune response through pro-inflammatory cytokines. Recently, *ERAP1* variants have been reported to alter levels of HLA-B27 free heavy chains on the cell surface. Altered enzyme activity could thus affect the rate at which MHC-peptide complexes fold within the ER and contribute to the UPR and ER stress. Malfunctioning of *ERAP1* could facilitate the accumulation of abnormally folded HLA-B27 in the ER, leading to the activation of the unfolded protein response and a downstream immune response, such as through the upregulation of IL-23.

In conclusion, it is highly likely that more than one of these hypotheses is involved in SpA pathogenesis. This is strengthened by the fact that in each of these hypotheses HLA-B27 plays a central role.

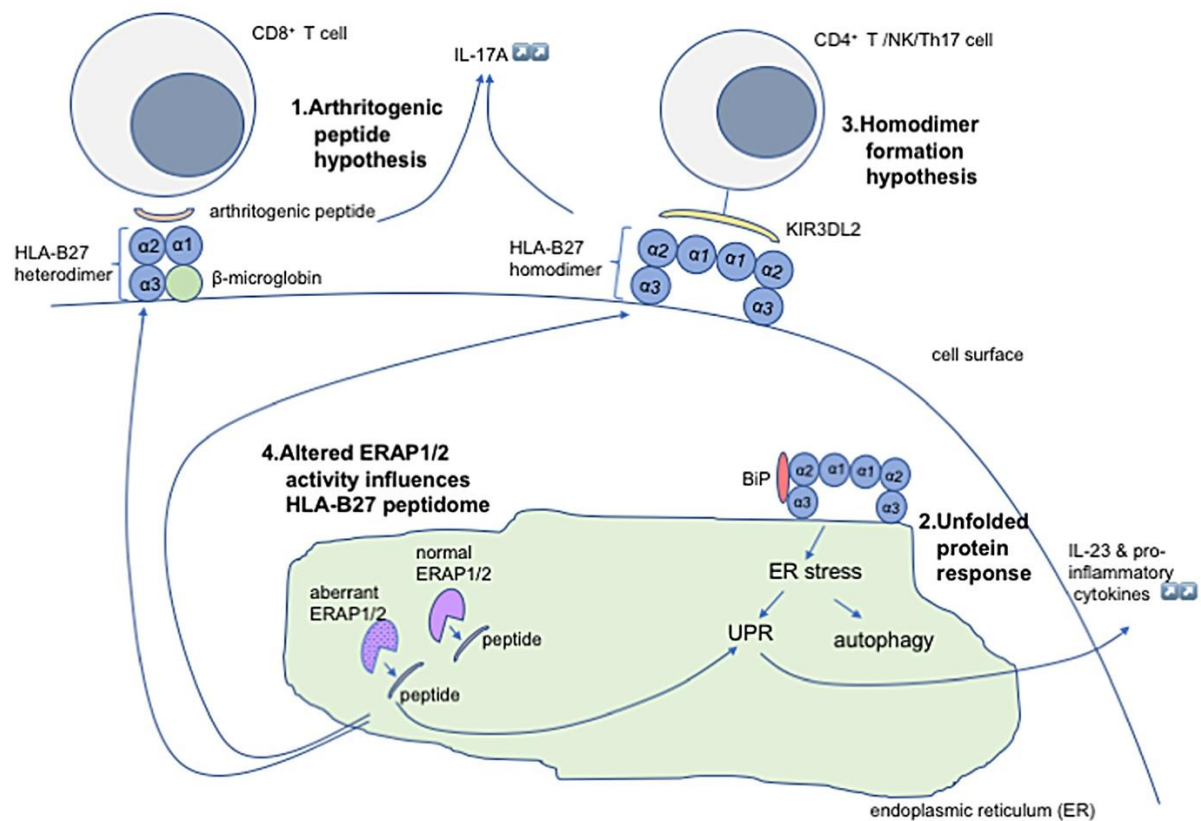


Figure 2. Illustration of the hypothesis for the pathogenic role of HLA-B*2705 molecules in spondyloarthritis. (1) Arthritogenic peptides displayed by properly folded HLA-B27 can be recognized by autoreactive CD8⁺ T cells, resulting in inflammation (2) Misfolded HLA-B27 chains and binding of BiP causes ER stress and activation of UPR, leading to increased production of IL-23 and other proinflammatory cytokines (3) Cell surface HLA homodimers interact with CD4⁺ T cells through innate immune receptors such as KIR3DL2 and facilitate cell-mediated autoimmune responses (4) Altered ERAP1 activity can result in changes in peptide processing with pathological consequences. ER, endoplasmic reticulum; ERAP1, ER aminopeptidases (ERAP)1; KIR3DL2, killer immunoglobulin-like receptor; UPR, unfolded protein response.

Author contribution

A.-S.; conceptualization, writing—original draft preparation, J. K.; writing—review and editing; program supervision; funding acquisition.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Zochling, J.; Brandt, J.; Braun, J. The current concept of spondyloarthritis with special emphasis on undifferentiated spondyloarthritis. *Rheumatology (Oxford)* **2005**, *44*, 1483-1491, doi:10.1093/rheumatology/kei047.
2. Khan, M.A. Polymorphism of HLA-B27: 105 subtypes currently known. *Curr Rheumatol Rep* **2013**, *15*, 362, doi:10.1007/s11926-013-0362-y.
3. Stolwijk, C.; Boonen, A.; van Tubergen, A.; Reveille, J.D. Epidemiology of spondyloarthritis. *Rheum Dis Clin North Am* **2012**, *38*, 441-476, doi:10.1016/j.rdc.2012.09.003.
4. Bakland, G.; Nossent, H.C. Epidemiology of Spondyloarthritis: A Review. *Current Rheumatology Reports* **2013**, *15*, 351, doi:10.1007/s11926-013-0351-1.
5. Braun, J.; Sieper, J. Ankylosing spondylitis. *The Lancet* **2007**, *369*, 1379-1390, doi:10.1016/S0140-6736(07)60635-7.
6. Gotch, F.; Rothbard, J.; Howland, K.; Townsend, A.; McMichael, A. Cytotoxic T lymphocytes recognize a fragment of influenza virus matrix protein in association with HLA-A2. *Nature* **1987**, *326*, 881-882, doi:10.1038/326881a0.

7. Townsend, A.R.M.; Rothbard, J.; Gotch, F.M.; Bahadur, G.; Wraith, D.; McMichael, A.J. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. *Cell* **1986**, *44*, 959-968, doi:[https://doi.org/10.1016/0092-8674\(86\)90019-X](https://doi.org/10.1016/0092-8674(86)90019-X).
8. Colbert, R.A.; Tran, T.M.; Layh-Schmitt, G. HLA-B27 misfolding and ankylosing spondylitis. *Mol Immunol* **2014**, *57*, 44-51, doi:10.1016/j.molimm.2013.07.013.
9. Trowsdale, J.; Knight, J.C. Major histocompatibility complex genomics and human disease. *Annu Rev Genomics Hum Genet* **2013**, *14*, 301-323, doi:10.1146/annurev-genom-091212-153455.
10. Bjorkman, P.; Parham, P. Structure, Function, and Diversity of Class I Major Histocompatibility Complex Molecules. *Annual review of biochemistry* **1990**, *59*, 253-288, doi:10.1146/annurev.bi.59.070190.001345.
11. Crux, N.; Elahi, S. Human Leukocyte Antigen (HLA) and Immune Regulation: How Do Classical and Non-Classical HLA Alleles Modulate Immune Response to Human Immunodeficiency Virus and Hepatitis C Virus Infections? *Frontiers in Immunology* **2017**, *8*, doi:10.3389/fimmu.2017.00832.
12. McMichael, A.; Bowness, P. HLA-B27: natural function and pathogenic role in spondyloarthritis. *Arthritis Res* **2002**, *4 Suppl 3*, S153-158, doi:10.1186/ar571.
13. Reveille, J.D. 57 - Spondyloarthritis. In *Clinical Immunology (Fifth Edition)*, Rich, R.R., Fleisher, T.A., Shearer, W.T., Schroeder, H.W., Frew, A.J., Weyand, C.M., Eds. Content Repository Only!: London, 2019; <https://doi.org/10.1016/B978-0-7020-6896-6.00057-0pp>. 769-787.
14. Hill Gaston, J.S.; Lillicrap, M.S. Arthritis associated with enteric infection. *Best Pract Res Clin Rheumatol* **2003**, *17*, 219-239, doi:10.1016/s1521-6942(02)00104-3.
15. Sieper, J.; Braun, J.; Kingsley, G.H. Report on the Fourth International Workshop on Reactive Arthritis. *Arthritis Rheum* **2000**, *43*, 720-734, doi:10.1002/1529-0131(200004)43:4<720::AID-ANR2>3.0.CO;2-8.

16. Granfors, K.; Jalkanen, S.; von Essen, R.; Lahesmaa-Rantala, R.; Isomäki, O.; Pekkola-Heino, K.; Merilahti-Palo, R.; Saario, R.; Isomäki, H.; Toivanen, A. Yersinia Antigens in Synovial-Fluid Cells from Patients with Reactive Arthritis. *New England Journal of Medicine* **1989**, *320*, 216-221, doi:10.1056/NEJM198901263200404.
17. Allen, R.L.; Bowness, P.; McMichael, A. The role of HLA-B27 in spondyloarthritis. *Immunogenetics* **1999**, *50*, 220-227, doi:10.1007/s002510050596.
18. Zhu, W.; He, X.; Cheng, K.; Zhang, L.; Chen, D.; Wang, X.; Qiu, G.; Cao, X.; Weng, X. Ankylosing spondylitis: etiology, pathogenesis, and treatments. *Bone Res* **2019**, *7*, 22, doi:10.1038/s41413-019-0057-8.
19. Oldstone, M.B.A. Molecular Mimicry as a Mechanism for the Cause and as a Probe Uncovering Etiologic Agent(S) of Autoimmune-Disease. *Current Topics in Microbiology and Immunology* **1989**, *145*, 127-135.
20. Grandon, B.; Rincheval-Arnold, A.; Jah, N.; Corsi, J.M.; Araujo, L.M.; Glatigny, S.; Prevost, E.; Roche, D.; Chiocchia, G.; Guenal, I., et al. HLA-B27 alters BMP/TGFbeta signalling in Drosophila, revealing putative pathogenic mechanism for spondyloarthritis. *Ann Rheum Dis* **2019**, *78*, 1653-1662, doi:10.1136/annrheumdis-2019-215832.
21. Pedersen, S.J.; Maksymowych, W.P. The Pathogenesis of Ankylosing Spondylitis: an Update. *Curr Rheumatol Rep* **2019**, *21*, 58, doi:10.1007/s11926-019-0856-3.
22. Garcia-Montoya, L.; Gul, H.; Emery, P. Recent advances in ankylosing spondylitis: understanding the disease and management. *F1000Res* **2018**, *7*, doi:10.12688/f1000research.14956.1.
23. Martin-Esteban, A.; Sanz-Bravo, A.; Guasp, P.; Barnea, E.; Admon, A.; de Castro, J.A.L. Separate effects of the ankylosing spondylitis associated ERAP1 and ERAP2 aminopeptidases determine the influence of their combined phenotype on the HLA-B*27 peptidome. *Journal of Autoimmunity* **2017**, *79*, 28-38, doi:10.1016/j.jaut.2016.12.008.

24. Babaie, F.; Mohammadi, H.; Hemmatzadeh, M.; Ebraze, M.; Torkamandi, S.; Yousefi, M.; Hajaliloo, M.; Rezaeiemanesh, A.; Salimi, S.; Salimi, R., et al. Evaluation of ERAP1 gene single nucleotide polymorphisms in immunomodulation of pro-inflammatory and anti-inflammatory cytokines profile in ankylosing spondylitis. *Immunol Lett* **2020**, *217*, 31-38, doi:10.1016/j.imlet.2019.10.016.
25. Stoll, M.L. Interactions of the innate and adaptive arms of the immune system in the pathogenesis of spondyloarthritis. *Clin Exp Rheumatol* **2011**, *29*, 322-330.
26. Cusick, M.F.; Libbey, J.E.; Fujinami, R.S. Molecular Mimicry as a Mechanism of Autoimmune Disease. *Clinical Reviews in Allergy & Immunology* **2012**, *42*, 102-111, doi:10.1007/s12016-011-8294-7.
27. Kuon, W.; Holzhutter, H.G.; Appel, H.; Grolms, M.; Kollnberger, S.; Traeder, A.; Henklein, P.; Weiss, E.; Thiel, A.; Lauster, R., et al. Identification of HLA-B27-restricted peptides from the *Chlamydia trachomatis* proteome with possible relevance to HLA-B27-associated diseases. *J Immunol* **2001**, *167*, 4738-4746, doi:10.4049/jimmunol.167.8.4738.
28. Ramos, M.; Lopez de Castro, J.A. HLA-B27 and the pathogenesis of spondyloarthritis. *Tissue Antigens* **2002**, *60*, 191-205, doi:10.1034/j.1399-0039.2002.600301.x.
29. Bowness, P. Hla-B27. *Annual Review of Immunology Vol 33* **2015**, *33*, 29-48, doi:10.1146/annurev-immunol-032414-112110.
30. Appel, H.; Kuon, W.; Kuhne, M.; Wu, P.H.; Kuhlmann, S.; Kollnberger, S.; Thiel, A.; Bowness, P.; Sieper, J. Use of HLA-B27 tetramers to identify low-frequency antigen-specific T cells in *Chlamydia*-triggered reactive arthritis. *Arthritis Research & Therapy* **2004**, *6*, R521-R534, doi:10.1186/ar1221.
31. Hermann, E.; Yu, D.T.Y.; Zumbuschfeld, K.H.M.; Fleischer, B. Hla-B27-Restricted Cd8 T-Cells Derived from Synovial-Fluids of Patients with Reactive Arthritis and Ankylosing-Spondylitis. *Lancet* **1993**, *342*, 646-650, doi:10.1016/0140-6736(93)91760-J.

32. Ugrinovic, S.; Mertz, A.; Wu, P.; Braun, J.; Sieper, J. A single nonamer from the Yersinia 60-kDa heat shock protein is the target of HLA-B27-restricted CTL response in Yersinia-induced reactive arthritis. *J Immunol* **1997**, *159*, 5715-5723.
33. Scofield, R.H.; Kurien, B.; Gross, T.; Warren, W.L.; Harley, J.B. HLA-B27 binding of peptide from its own sequence and similar peptides from bacteria: implications for spondyloarthropathies. *Lancet* **1995**, *345*, 1542-1544, doi:10.1016/s0140-6736(95)91089-1.
34. Ewing, C.; Ebringer, R.; Tribbick, G.; Geysen, H.M. Antibody activity in ankylosing spondylitis sera to two sites on HLA B27.1 at the MHC groove region (within sequence 65-85), and to a Klebsiella pneumoniae nitrogenase reductase peptide (within sequence 181-199). *J Exp Med* **1990**, *171*, 1635-1647, doi:10.1084/jem.171.5.1635.
35. Ben Dror, L.; Barnea, E.; Beer, I.; Mann, M.; Admon, A. The HLA-B*2705 peptidome. *Arthritis Rheum* **2010**, *62*, 420-429, doi:10.1002/art.27257.
36. Fiorillo, M.T.; Maragno, M.; Butler, R.; Dupuis, M.L.; Sorrentino, R. CD8(+) T-cell autoreactivity to an HLA-B27-restricted self-epitope correlates with ankylosing spondylitis. *J Clin Invest* **2000**, *106*, 47-53, doi:10.1172/JCI9295.
37. Syrbe, U.; Sieper, J. Chapter 36 - Spondyloarthritides. In *The Autoimmune Diseases (Sixth Edition)*, Rose, N.R., Mackay, I.R., Eds. Academic Press: 2020; <https://doi.org/10.1016/B978-0-12-812102-3.00036-1pp>. 691-701.
38. Schittenhelm, R.B.; Sian, T.C.; Wilmann, P.G.; Dudek, N.L.; Purcell, A.W. Revisiting the arthritogenic peptide theory: quantitative not qualitative changes in the peptide repertoire of HLA-B27 allotypes. *Arthritis Rheumatol* **2015**, *67*, 702-713, doi:10.1002/art.38963.
39. Babaie, F.; Hasankhani, M.; Mohammadi, H.; Safarzadeh, E.; Rezaiemanesh, A.; Salimi, R.; Baradaran, B.; Babaloo, Z. The role of gut microbiota and IL-23/IL-17 pathway in ankylosing spondylitis immunopathogenesis: New insights and updates. *Immunol Lett* **2018**, *196*, 52-62, doi:10.1016/j.imlet.2018.01.014.

40. Mear, J.P.; Schreiber, K.L.; Munz, C.; Zhu, X.M.; Stevanovic, S.; Rammensee, H.G.; Rowland-Jones, S.L.; Colbert, R.A. Misfolding of HLA-B27 as a result of its B pocket suggests a novel mechanism for its role in susceptibility to spondyloarthropathies. *Journal of Immunology* **1999**, *163*, 6665-6670.
41. Kenna, T.J.; Robinson, P.C.; Haroon, N. Endoplasmic reticulum aminopeptidases in the pathogenesis of ankylosing spondylitis. *Rheumatology* **2015**, *54*, 1549-1556, doi:10.1093/rheumatology/kev218.
42. Ambarus, C.A.; Yeremenko, N.; Baeten, D.L. Altered cytokine expression by macrophages from HLA-B27-positive spondyloarthritis patients without evidence of endoplasmic reticulum stress. *Rheumatol Adv Pract* **2018**, *2*, rky014, doi:10.1093/rap/rky014.
43. Smith, J.A. Regulation of Cytokine Production by the Unfolded Protein Response; Implications for Infection and Autoimmunity. *Front Immunol* **2018**, *9*, 422, doi:10.3389/fimmu.2018.00422.
44. Busch, R.; Kollnberger, S.; Mellins, E.D. HLA associations in inflammatory arthritis: emerging mechanisms and clinical implications. *Nat Rev Rheumatol* **2019**, *15*, 364-381, doi:10.1038/s41584-019-0219-5.
45. Navid, F.; Layh-Schmitt, G.; Sikora, K.A.; Cougnoux, A.; Colbert, R.A. The Role of Autophagy in the Degradation of Misfolded HLA-B27 Heavy Chains. *Arthritis Rheumatol* **2018**, *70*, 746-755, doi:10.1002/art.40414.
46. Khare, S.D.; Luthra, H.S.; David, C.S. Spontaneous inflammatory arthritis in HLA-B27 transgenic mice lacking beta 2-microglobulin: a model of human spondyloarthropathies. *J Exp Med* **1995**, *182*, 1153-1158, doi:10.1084/jem.182.4.1153.
47. Ramos, M.; de Castro, J.A.L. HLA-B27 and the pathogenesis of spondyloarthritis. *Tissue Antigens* **2002**, *60*, 191-205, doi:DOI 10.1034/j.1399-0039.2002.600301.x.
48. Ebringer, A. The Cross-Tolerance Hypothesis, Hla-B27 and Ankylosing-Spondylitis. *Brit J Rheumatol* **1983**, *22*, 53-66.

49. Turner, M.J.; Delay, M.L.; Bai, S.; Klenk, E.; Colbert, R.A. HLA-B27 up-regulation causes accumulation of misfolded heavy chains and correlates with the magnitude of the unfolded protein response in transgenic rats: Implications for the pathogenesis of spondylarthritis-like disease. *Arthritis Rheum* **2007**, *56*, 215-223, doi:10.1002/art.22295.
50. Delay, M.L.; Turner, M.J.; Klenk, E.I.; Smith, J.A.; Sowders, D.P.; Colbert, R.A. HLA-B27 Misfolding and the Unfolded Protein Response Augment Interleukin-23 Production and Are Associated With Th17 Activation in Transgenic Rats. *Arthritis Rheum-Us* **2009**, *60*, 2633-2643, doi:10.1002/art.24763.
51. Tran, T.M.; Dorris, M.L.; Satumtira, N.; Richardson, J.A.; Hammer, R.E.; Shang, J.; Taurog, J.D. Additional human beta(2)-microglobulin curbs HLA-B27 misfolding and promotes arthritis and spondylitis without colitis in male HLA-B27-transgenic rats. *Arthritis Rheum-Us* **2006**, *54*, 1317-1327, doi:10.1002/art.21740.
52. Campbell, E.C.; Fettke, F.; Bhat, S.; Morley, K.D.; Powis, S.J. Expression of MHC class I dimers and ERAP1 in an ankylosing spondylitis patient cohort. *Immunology* **2011**, *133*, 379-385, doi:10.1111/j.1365-2567.2011.03453.x.
53. Ciccia, F.; Accardo-Palumbo, A.; Rizzo, A.; Guggino, G.; Raimondo, S.; Giardina, A.; Cannizzaro, A.; Colbert, R.A.; Alessandro, R.; Triolo, G. Evidence that autophagy, but not the unfolded protein response, regulates the expression of IL-23 in the gut of patients with ankylosing spondylitis and subclinical gut inflammation. *Annals of the Rheumatic Diseases* **2014**, *73*, 1566-1574, doi:10.1136/annrheumdis-2012-202925.
54. Rezaeiemanesh, A.; Mahmoudi, M.; Amirzargar, A.A.; Vojdani, M.; Jamshidi, A.R.; Nicknam, M.H. Ankylosing spondylitis M-CSF-derived macrophages are undergoing unfolded protein response (UPR) and express higher levels of interleukin-23. *Mod Rheumatol* **2017**, *27*, 862-867, doi:10.1080/14397595.2016.1259716.

55. Taurog, J.D.; Dorris MI Fau - Satumtira, N.; Satumtira N Fau - Tran, T.M.; Tran Tm Fau - Sharma, R.; Sharma R Fau - Dressel, R.; Dressel R Fau - van den Brandt, J.; van den Brandt J Fau - Reichardt, H.M.; Reichardt, H.M. Spondylarthritis in HLA-B27/human beta2-microglobulin-transgenic rats is not prevented by lack of CD8.
56. Boyle, L.H.; Hill Gaston, J.S. Breaking the rules: the unconventional recognition of HLA-B27 by CD4+ T lymphocytes as an insight into the pathogenesis of the spondyloarthropathies. *Rheumatology* **2003**, *42*, 404-412, doi:10.1093/rheumatology/keg097.
57. Kollnberger, S.; Bird, L.; Sun, M.-Y.; Retiere, C.; Braud, V.M.; McMichael, A.; Bowness, P. Cell-surface expression and immune receptor recognition of HLA-B27 homodimers. *Arthritis & Rheumatism* **2002**, *46*, 2972-2982, doi:10.1002/art.10605.
58. Antoniou, A.N.; Ford, S.; Taurog, J.D.; Butcher, G.W.; Powis, S.J. Formation of HLA-B27 homodimers and their relationship to assembly kinetics. *Journal of Biological Chemistry* **2004**, *279*, 8895-8902, doi:10.1074/jbc.M311757200.
59. Brown, M.A. Solving the pathogenesis of ankylosing spondylitis. *Clin Immunol* **2018**, *186*, 46-50, doi:10.1016/j.clim.2017.07.011.
60. Bowness, P.; Ridley, A.; Shaw, J.; Chan, A.T.; Wong-Baeza, I.; Fleming, M.; Cummings, F.; McMichael, A.; Kollnberger, S. Th17 cells expressing KIR3DL2+ and responsive to HLA-B27 homodimers are increased in ankylosing spondylitis. *J Immunol* **2011**, *186*, 2672-2680, doi:10.4049/jimmunol.1002653.
61. Chen, L.; Ridley, A.; A, H.; Al-Mossawi, H.; Bunting, H.; Georgiadis, D.; Chan, A.; Kollnberger, S.; Bowness, P. Silencing or inhibition of endoplasmic reticulum aminopeptidase 1 (ERAP1) suppresses free heavy chain expression and Th17 responses in ankylosing spondylitis. *Annals of the Rheumatic Diseases* **2015**, *75*, doi:10.1136/annrheumdis-2014-206996.
62. Babaie, F.; Ebrazeh, M.; Hemmatzadeh, M.; Sadat Mohammadi, F.; Gowhari Shabgah, A.; Hajaliloo, M.; Ebrahimi, A.A.; Shirafkan, N.; Azizi, G.;

- Mohammadi, H., et al. Association analysis of ERAP1 gene single nucleotide polymorphism in susceptibility to ankylosing spondylitis in Iranian population. *Immunol Lett* **2018**, *201*, 52-58, doi:10.1016/j.imlet.2018.11.002.
63. Giles, J.; Shaw J Fau - Piper, C.; Piper C Fau - Wong-Baeza, I.; Wong-Baeza I Fau - McHugh, K.; McHugh K Fau - Ridley, A.; Ridley A Fau - Li, D.; Li D Fau - Lenart, I.; Lenart I Fau - Antoniou, A.N.; Antoniou An Fau - DiGleria, K.; DiGleria K Fau - Kuroki, K., et al. HLA-B27 homodimers and free H chains are stronger ligands for leukocyte Ig-like receptor B2 than classical HLA class I. D - NLM: EMS52285 EDAT- 2012/05/18 06:00 MHDA- 2012/08/15 06:00 CRDT- 2012/05/18 06:00 PHST- 2012/05/18 06:00 [entrez] PHST- 2012/05/18 06:00 [pubmed] PHST- 2012/08/15 06:00 [medline] AID - jimmunol.1102711 [pii] AID - 10.4049/jimmunol.1102711 [doi] PST - ppublish, doi:D - NLM: EMS52285 EDAT- 2012/05/18 06:00 MHDA- 2012/08/15 06:00 CRDT- 2012/05/18 06:00 PHST- 2012/05/18 06:00 [entrez] PHST- 2012/05/18 06:00 [pubmed] PHST- 2012/08/15 06:00 [medline] AID - jimmunol.1102711 [pii] AID - 10.4049/jimmunol.1102711 [doi] PST - ppublish.
64. Chan, A.T.; Kollnberger Sd Fau - Wedderburn, L.R.; Wedderburn Lr Fau - Bowness, P.; Bowness, P. Expansion and enhanced survival of natural killer cells expressing the killer immunoglobulin-like receptor KIR3DL2 in spondylarthritis.
65. Cauli, A.; Shaw, J.; Giles, J.; Hatano, H.; Rysnik, O.; Payeli, S.; McHugh, K.; Dessole, G.; Porru, G.; Desogus, E., et al. The arthritis-associated HLA-B*27:05 allele forms more cell surface B27 dimer and free heavy chain ligands for KIR3DL2 than HLA-B*27:09. *Rheumatology (Oxford, England)* **2013**, *52*, 1952-1962, doi:10.1093/rheumatology/ket219.
66. Lim Kam Sian, T.C.C.; Indumathy, S.; Halim, H.; Greule, A.; Cryle, M.J.; Bowness, P.; Rossjohn, J.; Gras, S.; Purcell, A.W.; Schittenhelm, R.B. Allelic association with ankylosing spondylitis fails to correlate with human leukocyte

- antigen B27 homodimer formation. *J Biol Chem* **2019**, *294*, 20185-20195, doi:10.1074/jbc.RA119.010257.
67. Evans, D.M.; Spencer, C.C.A.; Pointon, J.J.; Su, Z.; Harvey, D.; Kochan, G.; Opperman, U.; Dilthey, A.; Pirinen, M.; Stone, M.A., et al. Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. *Nature Genetics* **2011**, *43*, 761-U767, doi:10.1038/ng.873.
68. Mahmoudi, M.; Jamshidi, A.R.; Amirzargar, A.A.; Farhadi, E.; Nourijelyani, K.; Fallahi, S.; Oraei, M.; Noori, S.; Nicknam, M.H. Association between Endoplasmic Reticulum Aminopeptidase-1 (ERAP-1) and Susceptibility to Ankylosing Spondylitis in Iran. *Iranian Journal of Allergy Asthma and Immunology* **2012**, *11*, 294-300.
69. Dashti, N.; Mahmoudi, M.; Aslani, S.; Jamshidi, A. HLA-B*27 subtypes and their implications in the pathogenesis of ankylosing spondylitis. *Gene* **2018**, *670*, 15-21, doi:<https://doi.org/10.1016/j.gene.2018.05.092>.
70. Davidson, S.I.; Wu X Fau - Liu, Y.; Liu Y Fau - Wei, M.; Wei M Fau - Danoy, P.A.; Danoy Pa Fau - Thomas, G.; Thomas G Fau - Cai, Q.; Cai Q Fau - Sun, L.; Sun L Fau - Duncan, E.; Duncan E Fau - Wang, N.; Wang N Fau - Yu, Q., et al. Association of ERAP1, but not IL23R, with ankylosing spondylitis in a Han Chinese population.
71. Burton, P.; Clayton, D.; Cardon, L.; Craddock, N.; Duncanson, A.; Kwiatkowski, D.; McCarthy, M.; Ouwehand, W.; Samani, N.; Todd, J., et al. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nature genetics* **2007**, *39*, 1329-1337, doi:10.1038/ng.2007.17.
72. Brown, M.A. Breakthroughs in genetic studies of ankylosing spondylitis. *Rheumatology* **2007**, *47*, 132-137, doi:10.1093/rheumatology/kem269.
73. Saric, T.; Chang, S.C.; Hattori, A.; York, I.A.; Markant, S.; Rock, K.L.; Tsujimoto, M.; Goldberg, A.L. An IFN-gamma-induced aminopeptidase in the ER, ERAP1,

- trims precursors to MHC class I-presented peptides. *Nat Immunol* **2002**, *3*, 1169-1176, doi:10.1038/ni859.
74. Saveanu, L.; van Endert, P. The role of insulin-regulated aminopeptidase in MHC class I antigen presentation. *Front Immunol* **2012**, *3*, 57, doi:10.3389/fimmu.2012.00057.
75. McHugh, K.; Bowness, P. The link between HLA-B27 and SpA--new ideas on an old problem. *Rheumatology (Oxford, England)* **2012**, *51*, 1529-1539, doi:10.1093/rheumatology/kes061.
76. Cui, X.L.; Hawari, F.; Alsaaty, S.; Lawrence, M.; Combs, C.A.; Geng, W.D.; Rouhani, F.N.; Miskinis, D.; Levine, S.J. Identification of ARTS-1 as a novel TNFR1-binding protein that promotes TNFR1 ectodomain shedding. *Journal of Clinical Investigation* **2002**, *110*, 515-526, doi:10.1172/Jci200213847.
77. Reveille, J.D. Genetics of spondyloarthritis—beyond the MHC. *Nature Reviews Rheumatology* **2012**, *8*, 296-304, doi:10.1038/nrrheum.2012.41.
78. Keidel, S.; Chen, L.; Pointon, J.; Wordsworth, P. ERAP1 and ankylosing spondylitis. *Curr Opin Immunol* **2013**, *25*, 97-102, doi:10.1016/j.coi.2012.11.002.
79. Kirino, Y.; Bertsias, G.; Ishigatsubo, Y.; Mizuki, N.; Tugal-Tutkun, I.; Seyahi, E.; Ozyazgan, Y.; Sacli, F.S.; Erer, B.; Inoko, H., et al. Genome-wide association analysis identifies new susceptibility loci for Behcet's disease and epistasis between HLA-B*51 and ERAP1. *Nat Genet* **2013**, *45*, 202-207, doi:10.1038/ng.2520.
80. Genetic Analysis of Psoriasis, C.; the Wellcome Trust Case Control, C.; Strange, A.; Capon, F.; Spencer, C.C.A.; Knight, J.; Weale, M.E.; Allen, M.H.; Barton, A.; Band, G., et al. A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nature genetics* **2010**, *42*, 985-990, doi:10.1038/ng.694.
81. Kochan, G.; Krojer, T.; Harvey, D.; Fischer, R.; Chen, L.; Vollmar, M.; von Delft, F.; Kavanagh, K.L.; Brown, M.A.; Bowness, P., et al. Crystal structures of the endoplasmic reticulum aminopeptidase-1 (ERAP1) reveal the molecular basis

- for N-terminal peptide trimming. *Proc Natl Acad Sci U S A* **2011**, *108*, 7745-7750, doi:10.1073/pnas.1101262108.
82. Li, L.; Batliwala, M.; Bouvier, M. ERAP1 enzyme-mediated trimming and structural analyses of MHC I-bound precursor peptides yield novel insights into antigen processing and presentation. *Journal of Biological Chemistry* **2019**, *294*, jbc.RA119.010102, doi:10.1074/jbc.RA119.010102.
83. Seregin, S.S.; Rastall, D.P.; Evnouchidou, I.; Aylsworth, C.F.; Quiroga, D.; Kamal, R.P.; Godbehere-Roosa, S.; Blum, C.F.; York, I.A.; Stratikos, E., et al. Endoplasmic reticulum aminopeptidase-1 alleles associated with increased risk of ankylosing spondylitis reduce HLA-B27 mediated presentation of multiple antigens. *Autoimmunity* **2013**, *46*, 497-508, doi:10.3109/08916934.2013.819855.
84. Tang, Y.; Yang, P.; Wang, F.; Xu, H.; Zong, S.Y. Association of polymorphisms in ERAP1 and risk of ankylosing spondylitis in a Chinese population. *Gene* **2018**, *646*, 8-11, doi:10.1016/j.gene.2017.12.050.
85. Wisniewski, A.; Kasprzyk, S.; Majorczyk, E.; Nowak, I.; Wilczynska, K.; Chlebicki, A.; Zon-Giebel, A.; Kusnierczyk, P. ERAP1-ERAP2 haplotypes are associated with ankylosing spondylitis in Polish patients. *Hum Immunol* **2019**, *80*, 339-343, doi:10.1016/j.humimm.2019.02.004.
86. Paladini, F.; Fiorillo, M.T.; Tedeschi, V.; D'Otolo, V.; Piga, M.; Cauli, A.; Mathieu, A.; Sorrentino, R. The rs75862629 minor allele in the endoplasmic reticulum aminopeptidases intergenic region affects human leucocyte antigen B27 expression and protects from ankylosing spondylitis in Sardinia. *Rheumatology (Oxford)* **2019**, *58*, 2315-2324, doi:10.1093/rheumatology/kez212.
87. Hammer, G.E.; Gonzalez, F.; Champsaur, M.; Cado, D.; Shastri, N. The aminopeptidase ERAAP shapes the peptide repertoire displayed by major histocompatibility complex class I molecules. *Nat Immunol* **2006**, *7*, 103-112, doi:10.1038/ni1286.
88. Haroon, N.; Tsui, F.; Chiu, B.; Tsui, H.; Inman, R. Serum Cytokine Receptors in Ankylosing Spondylitis: Relationship to Inflammatory Markers and

- Endoplasmic Reticulum Aminopeptidase Polymorphisms. *The Journal of rheumatology* **2010**, *37*, 1907-1910, doi:10.3899/jrheum.100019.
89. Chen, B.; Li, D.; Xu, W. Association of ankylosing spondylitis with HLA-B27 and ERAP1: pathogenic role of antigenic peptide. *Med Hypotheses* **2013**, *80*, 36-38, doi:10.1016/j.mehy.2012.10.003.
90. Gill, T.; Asquith, M.; Brooks, S.R.; Rosenbaum, J.T.; Colbert, R.A. Effects of HLA-B27 on Gut Microbiota in Experimental Spondyloarthritis Implicate an Ecological Model of Dysbiosis. *Arthritis Rheumatol* **2018**, *70*, 555-565, doi:10.1002/art.40405.
91. Antoniou, A.N.; Lenart, I.; Kriston-Vizi, J.; Iwawaki, T.; Turmaine, M.; McHugh, K.; Ali, S.; Blake, N.; Bowness, P.; Bajaj-Elliott, M., et al. Salmonella exploits HLA-B27 and host unfolded protein responses to promote intracellular replication. *Ann Rheum Dis* **2019**, *78*, 74-82, doi:10.1136/annrheumdis-2018-213532.
92. Ansalone, C.; Utriainen, L.; Milling, S.; Goodyear, C.S. Role of Gut Inflammation in Altering the Monocyte Compartment and Its Osteoclastogenic Potential in HLA-B27-Transgenic Rats. *Arthritis Rheumatol* **2017**, *69*, 1807-1815, doi:10.1002/art.40154.
93. Ombrello, M.J.; Remmers, E.F.; Tachmazidou, I.; Grom, A.; Foell, D.; Haas, J.P.; Martini, A.; Gattorno, M.; Ozen, S.; Prahalad, S., et al. HLA-DRB1*11 and variants of the MHC class II locus are strong risk factors for systemic juvenile idiopathic arthritis. *Proc Natl Acad Sci U S A* **2015**, *112*, 15970-15975, doi:10.1073/pnas.1520779112.
94. Lin, P.; Bach, M.; Asquith, M.; Lee, A.Y.; Akileswaran, L.; Stauffer, P.; Davin, S.; Pan, Y.; Cambronne, E.D.; Dorris, M., et al. HLA-B27 and human beta2-microglobulin affect the gut microbiota of transgenic rats. *PLoS One* **2014**, *9*, e105684, doi:10.1371/journal.pone.0105684.

95. Rosenbaum, J.T.; Davey, M.P. Time for a gut check: evidence for the hypothesis that HLA-B27 predisposes to ankylosing spondylitis by altering the microbiome. *Arthritis Rheum* **2011**, *63*, 3195-3198, doi:10.1002/art.30558.
96. Asquith, M.J.; Stauffer, P.; Davin, S.; Mitchell, C.; Lin, P.; Rosenbaum, J.T. Perturbed Mucosal Immunity and Dysbiosis Accompany Clinical Disease in a Rat Model of Spondyloarthritis. *Arthritis Rheumatol* **2016**, *68*, 2151-2162, doi:10.1002/art.39681.
97. Sartor, R.B.; Wu, G.D. Roles for Intestinal Bacteria, Viruses, and Fungi in Pathogenesis of Inflammatory Bowel Diseases and Therapeutic Approaches. *Gastroenterology* **2017**, *152*, 327-339 e324, doi:10.1053/j.gastro.2016.10.012.
98. Rizzo, A.; Ferrante, A.; Guggino, G.; Ciccia, F. Gut inflammation in spondyloarthritis. *Best Pract Res Clin Rheumatol* **2017**, *31*, 863-876, doi:10.1016/j.berh.2018.08.012.
99. Reid, G.; Younes, J.A.; Van der Mei, H.C.; Gloor, G.B.; Knight, R.; Busscher, H.J. Microbiota restoration: natural and supplemented recovery of human microbial communities. *Nat Rev Microbiol* **2011**, *9*, 27-38, doi:10.1038/nrmicro2473.
100. Mielants, H.; Veys, E.M.; De Vos, M.; Cuvelier, C. Increased intestinal permeability in ankylosing spondylitis. *Gut* **1992**, *33*, 1150, doi:10.1136/gut.33.8.1150.
101. Costello, M.E.; Ciccia, F.; Willner, D.; Warrington, N.; Robinson, P.C.; Gardiner, B.; Marshall, M.; Kenna, T.J.; Triolo, G.; Brown, M.A. Brief Report: Intestinal Dysbiosis in Ankylosing Spondylitis. *Arthritis Rheumatol* **2015**, *67*, 686-691, doi:10.1002/art.38967.
102. Zhang, L.; Hu, Y.; Xu, Y.; Li, P.; Ma, H.; Li, X.; Li, M. The correlation between intestinal dysbiosis and the development of ankylosing spondylitis. *Microbial Pathogenesis* **2019**, *132*, 188-192, doi:<https://doi.org/10.1016/j.micpath.2019.04.038>.

103. Breban, M.; Tap, J.; Leboime, A.; Said-Nahal, R.; Langella, P.; Chiocchia, G.; Furet, J.P.; Sokol, H. Faecal microbiota study reveals specific dysbiosis in spondyloarthritis. *Ann Rheum Dis* **2017**, *76*, 1614-1622, doi:10.1136/annrheumdis-2016-211064.
104. Wen, C.; Zheng, Z.; Shao, T.; Liu, L.; Xie, Z.; Le Chatelier, E.; He, Z.; Zhong, W.; Fan, Y.; Zhang, L., et al. Quantitative metagenomics reveals unique gut microbiome biomarkers in ankylosing spondylitis. *Genome Biol* **2017**, *18*, 142, doi:10.1186/s13059-017-1271-6.
105. Yin, J.; Sternes, P.R.; Wang, M.; Song, J.; Morrison, M.; Li, T.; Zhou, L.; Wu, X.; He, F.; Zhu, J., et al. Shotgun metagenomics reveals an enrichment of potentially cross-reactive bacterial epitopes in ankylosing spondylitis patients, as well as the effects of TNFi therapy upon microbiome composition. *Annals of the Rheumatic Diseases* **2020**, *79*, 132, doi:10.1136/annrheumdis-2019-215763.