Ankylosing Spondylitis: A Multi-Factorial Autoimmune Disease. MHC Class I, Antigen Presentation and others to Blame

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Abstract

Ankylosing spondylitis (AS) is a chronic systemic inflammatory disorder. One major histocompatibility complex gene, HLA-B27, is the strongest known risk factor associated with AS. Some of the unique features of HLA-B27 have led to different hypotheses to explain the mechanisms underlying the association between HLA-B27 and AS pathogenesis. Additionally, other MHC factors, non-MHC factors and environmental factors have also been implicated in AS susceptibility. This review highlights the different HLA -B27 hypotheses, as well as the roles of some of the other factors in determining AS susceptibility. It is evident that, individually, none of these factors can take complete credit for causing AS since it is a multi-factorial autoimmune disease.

Keywords: Ankylosing spondylitis; Systemic inflammatory disorder; Autoimmune disease; Human immunodeficiency virus; Multi-factorial disease

Abbreviations: AS: Ankylosing Spondylitis; SPA: Spondyloarthropathies; MHC-I: Class I Major Histocompatibility Complex; P2: Side Chain of Residue 2; CTL: Cytotoxic CD8⁺ T cells; ER: Endoplasmic Reticulum; β2m: microglobulin β2; HC: Heavy Chain; UPR: Unfolding Protein Response; Tregs: Regulatory T cells; SNPs: Single Nucleotide Polymorphisms.

Introduction

Ankylosing spondylitis is a chronic systemic inflammatory disorder that primarily involves the sacroiliac joints and axial skeleton. It is part of the group of spondyloarthropathies (SpA), which is one of the most frequently occurring groups of inflammatory rheumatic disorders [1]. Patients are typically between ages 20 and 40 [2] and the most common symptoms are fatigue, inflammatory back pain, and peripheral enthesitis and arthritis; extra-articular manifestations, such as uveitis and diseases involving the pulmonary, cardiovascular, renal, neurological, or gastrointestinal systems, may also be present [3-5].

Although the etiology of AS is not completely understood, it is clear that both environmental and genetic factors contribute to the disease. As evidence of the importance of genetics, the class I major histocompatibility complex molecules (MHC-I) appear to play the most significant role in AS susceptibility [6]. The strongest association is with HLA-B27; approximately 90% of AS patients are HLA-B27 positive [7]. In fact, HLA-B27 imparts the largest relative risk of developing an autoimmune disease of any MHC-linked autoimmune disease. HLA-B27 was the first predisposing allele found in all forms of SpA more than 40 years ago [8]. A significant association was also found in a very small cohort, between AS and B*14, where 62.5% of the AS patients were HLA-B14⁺, but only 2% of the healthy donors carried this allotype [9]. Apart from HLA-B27 and HLA-B14, -B60 (a split antigen of -B40) and -B38 and -B39 (split antigens of -B16) have also been linked to AS [10].

The fact that only 1-2% of the HLA-B27 positive population develops AS [1,11], suggests that other factors also contribute to the pathogenesis of AS. Erap1, a gene involved in MHC-I antigen presentation – the process in which peptides are presented to CD8⁺ T cells – has been implicated, which suggests that the MHC-I pathway is highly important in AS

pathogenesis. This makes it likely that other genes involved in the MHC-I pathway could be important in the susceptibility of the disease, as well. Also, genome-wide association studies have implicated over 30 genes in susceptibility to AS [12]. Non-MHC-I genetic factors, such as IL-1A, IL-23R, also contribute to AS susceptibility [13] but, in this review, I will mainly focus on how MHC-I presentation contributes to the pathogenesis of AS. Also, studies of identical twin-pairs revealed a high concordance rate among siblings, but it is not 100% [14]. Therefore, environmental or epigenetic factors must contribute. Among these, the microbiota has been also recently involved as another important factor in the susceptibility to the AS.

This review summarizes the extensive literature on how the MHC-I molecule, antigenic peptides, other antigen presentation machinery, and several different pathogens can all influence the development of AS, together with other factors not involved in antigen presentation. This review also evaluates the different hypotheses (Figure 1), which have been proposed as an attempt to explain the mechanisms underlying the association between HLA-B27 and AS pathogenesis. Individually, no single molecule, factor or pathogen can take the credit for causing AS – it is clearly a multi-factorial disease.

HLA-B27

HLA-B27: The good, the bad and the ugly

In 1973, the discovery of a correlation between HLA-B27 and AS was the first time an inflammatory disease was shown to be associated to a HLA haplotype [7]. Since then, a number of other HLA-B27 syndromes have been discovered, including acute anterior uveitis, reactive arthritis (ReA), inflammatory bowel disease, and psoriatic arthritis. Yet, inheritance of HLA-B27 is not exclusively bad. In fact, several studies have shown that
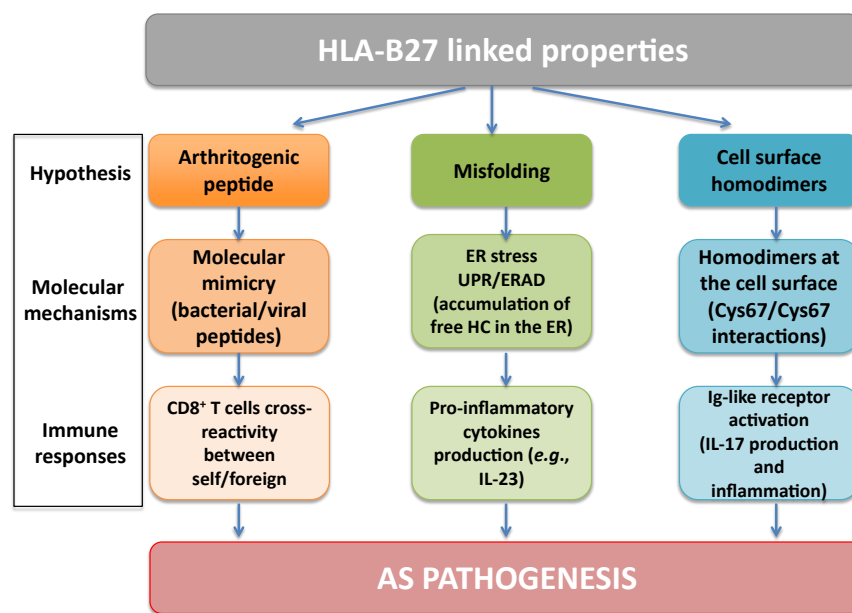
Figure 1: Flowchart showing the different hypotheses explaining the association with AS: HLA-B27 is a peculiar molecule with intrinsic properties that have led to different hypotheses trying to explain the association of this MHC-I molecule with AS: As peptide presenting molecule, HLA-B27 has the potential to present arthritogenic peptides. HLA-B27 tends to misfold in the ER and free HC tends to accumulate, leading to ER stress and the UPR/ERAD responses and production of pro-inflammatory cytokines. Also, HLA-B27 can form cell surface homodimers, which can be recognized by Ig-like receptors, followed by the stimulation of IL-17 production, leading to inflammation and, therefore pathogenesis. A detailed explanation of these hypotheses is given in the first sections of this review.

HLA-B27 has a protective role in human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections, where possession of HLA-B27 associated strongly with the absence of long term progression in HIV infection and spontaneous clearance of HCV [15,16]. HLA-B27-mediated protection is probably a combination of multiple viral and immunologic mechanisms, some of which may also be involved in AS pathogenesis.

HLA-B27 Subtypes: Differential peptide specificity and association to AS

HLA-B27 is an MHC-I molecule whose principal function is to present peptides, mostly from endogenous proteins, at the cell surface for CD8+ T cells. To date, close to 150 subtypes of HLA-B27 have been identified (http://www.ebi.ac.uk/ipd/imgt/hla/allele.html). The subtypes differ in their peptide-binding specificity, which is defined by the peptide-binding groove of this MHC-I molecule. The peptide-binding groove is arranged by the α1α2 and domains of MHC -I: it is high polymorphic [17] and consists of six side pockets (A-F) [18,19] that accommodate the bound peptide's side-chains [17]. Of particular importance are pocket B, which accommodates the side chain of residue 2 (P2), pocket F, which accommodates the C-terminal residue (usually P9) and pockets D and E which bind the side chains of residues 3 and 7 of the peptides, respectively. These pockets are usually very restrictive to an amino acid residue or a group of residues that are bound. These residues interact strongly with these pockets of the MHC-class I molecule and are often referred to as the "anchor residues" because they confer some degree of specificity to their associated MHC-I molecule. The rest of the residues in the peptide interact weakly with the MHC-class I molecule. These interactions are not with side-chains allowing the side-chains of these residues to be exposed on the molecular surface for the recognition by the TCR. HLA-B27 has a strong preference for peptides containing arginine at P2 [20], although glutamine was also found to reside in P2 in 3% of HLA-B27 ligands [21]. While the C-terminal residue is the second-most important anchor residue, it is not as conserved as P2; HLA-B27 ligands may possess a basic, aliphatic or aromatic residue at this position [22].

Each of the subtypes has a different degree of association to AS (Table 1). HLA-B*2705, -B*2704, and - B*2707 are linked to AS [23], while HLA-B*2706 and HLA-B*2709 are the most weakly associated with the disease [24-27]. These natural variants differ from the B*2705 prototype by one or a few amino acid residues, mostly at positions 114 and 116, and their different degrees of association to AS could be due to the differential peptide presentation. Differences in the amino-acid residues: 114 and 116, placed at the base of the peptide-binding groove are critical. The amino acid 116 lies at the bottom of the F pocket, interacting with the side chain of the peptide C-terminus and the amino acid 114 lies at the bottom of the pocket D, interacting with residue 3 of the peptide [28]. B*2709 differs from B*2705 at position 116 by a single amino acid, aspartic acid (D) to histidine (H) [29]. B*2704 and B*2706 differ by only two amino acid changes. B*2704 has histidine (H) at 114 and aspartic acid (D) at 116, while B*2706 has aspartic acid (D) at 114 and tyrosine (Y) at 116 [30-32]. The AS-associated subtype B*2707 has a tyrosine at position 116 [33]. Several studies have investigated the features of the

Subtype	AS*	
	Associated	Non-associated
HLA-B*2704	✓	
HLA-B*2705	✓	
HLA-B*2706		✓
HLA-B*2707(*)	✓	
HLA-B*2709(*)	✓	✓
HLA-B*1402		✓
HLA-B*1403	✓	

Table 1: Summary of the subtypes and their different association with AS* (*)Conflict with their association to disease (explained in detail in the review), AS* (Ankylosing spondylitis)

peptide-binding groove and the peptide pools presented by the HLA -B27 subtypes, reasoning that the peptides bound to the associated subtypes would have different features from the ones, which bind the non-associated subtypes. In these studies, systematic comparisons of pair-wise associated and non-associated subtypes (based on populations expressing these subtypes) were conducted in order to determine which peptide ligands are presented by the subtypes associated with AS. These studies indicated that these subtypes differentially associated to AS share about 80% of their peptide repertoires, and also bind some different ligands showing specific motifs in the AS-associated subtypes. Therefore, there are some shared and unique features of the peptides bound by these subtypes which could correlate peptide presentation and pathogenesis of the disease [34-37]. In summary, non-associated subtypes had a high restriction for non-polar C-terminal residues, including aliphatic ones and phenylalanine as compared to the associated ones, which are able to bind tyrosine. However, this straightforward correlation has been challenged by the AS-associated B*2707 subtype, in which, no peptide with tyrosine in the C-terminal was found [33]. In a more recent and a complete study of the seven major HLA-B27 subtypes; peptide-bound repertoires and peptides features, together with molecular stability were examined [38]. The results showed that peptides derived from AS-associated subtypes had more diverse C-terminal residues than the non-AS associated subtypes and this goes with what was shown in previous studies. Also, the residue 116 showed up as a very important feature in defining the peptide binding, folding and thermodynamic properties of the different subtypes. Subtypes associated to AS seem to bind better epitopes directly produced in the cytosol and they were more influenced by the protease ERAP1 (see MHC class I pathway section).

B*2709 was identified only in 20% of the B27 positive population in Sardinia and in 3% of the population in continental Italy, but in none of the AS patients or SpA patients [39]. However, B*2709 was found later on in some SpA patients, including Tunisians, questioning its 'non-association with AS [40-42].

The weak association between AS and HLA-B*2709, as compared to HLA-B*2705, was suggested to be in part due to a limited number of natural ligands bound, exclusively, by this allotype [36]. HLA-B*2709 presents a restriction in the C-terminal residue bound, imposed by its polymorphism, where mostly all the peptides bound to this subtype had aliphatic and phenylalanine residues in their C-terminus. All of the HLA-B*2705-specific peptides possessed arginine, lysine, or tyrosine at P9. These data support the existence of some specific peptides bound to HLA-B*2705 which represent a limited set of its bound peptide repertoire with potential to trigger AS. A study in Sardinia showed that 2 distinct haplotypes (i.e., blocks of genes in linkage disequilibrium (LD)) that are transmitted together: A2; B27; Cw2; DR16 which harbors the AS associated B27 alleles and A32 or A30; B*2709; Cw1; DR12, harboring the non-associated HLA-B*2709 allele [43]. These findings make feasible that other genes within the HLA region, besides HLA-B27, may play some role in conferring susceptibility to AS. Thus, an alternative explanation for the absence of association between B*2709 and AS in Sardinia, could be that other alleles of the nearby gene(s) that are in LD with B*2709 confer protection from SpA development [44]. A recent study comparing peptide repertoires of the 8 most frequent HLA-B27 subtypes has revealed that quantitative changes in the peptidomes are also important for the association with AS [45]. The authors used the targeted approach of multiple reactions monitoring (MRM) mass spectrometry (MS) to precisely look at low-abundance peptides over the different HLA-B27 allotypes. This approach allowed the detection of reduced levels of tyrosine as a residue bound to the C-terminal in B*2709, supporting the previous finding that this non-associated subtype could bind only two peptides with arginine or tyrosine in the C-terminal [36]. In part, this finding supports the reason why B*2709 was previously related to undifferentiated SpA (uSpA) [46],

where the manifestation of the disease does not involve the axial skeleton but the patients show peripheral manifestations of SpA. These quantitative differences are important because they could be driving the progression of the disease and, they may set up the threshold of how much of the antigen is needed for auto reactive T cells to get selected and activated [45]. It would be helpful to use this approach to re-evaluate some of the older, less quantitative data regarding the HLA-B27 subtypes. For example, re-examining the peptide pool bound to B*2707 would determine whether there are even a few peptides with tyrosine at the C-terminal.

Therefore, this differential peptide binding may explain the possible existence of arthritogenic peptides (see below) causing AS. However, a more refine idea emerged from the elegant x-ray crystallography structural studies of B*2705 and B*2709 bound to a self-peptide derived from the vasoactive intestinal peptide, type 1 receptor (pVIPR; sequence RRKWRRWHL) [47]. pVIPR was displayed in 2 different conformations when bound to B*2705. In one conformation, the peptide and the heavy chain of the HLA-B27 molecule are bound by drastically different interactions, as opposed to the conventional conformation, exclusively found in the case of B*2709. This led to the speculation that the non-conventional conformation can alter the potential antigenic surface presented to the CD8+ T cells and this might generate auto reactivity. However, the idea of the contribution of dual conformation peptides to the susceptibility of the disease was challenged by the extended structural analysis of the B*2704 and the B*2706 subtypes [48]. In this study, the dual conformation was observed for the non-disease associated B*2706. In this study, the authors also probed the dynamics of these HLA-B*27 molecules using isotope-edited infrared (IR) spectroscopy, and including B*2705 and B*2709 as well. Rather than a dual conformation, the results demonstrated that the disease-associated subtypes B*2704 and B*2705 have a higher conformational flexibility. The heavy chain of the B*2705 complex had already shown an increased conformational flexibility compared to B*2709 heavy chain, in a previous study [49].

The Arthritogenic Peptide Hypothesis and AS

Based on the canonical function of HLA-B27, the "arthritogenic peptide" hypothesis suggests that the arthritis in AS patients is a consequence of HLA-B27 presenting joint-specific peptides to autoreactive CD8+ T cells. Molecular mimicry, or cross-reactivity between bacterial antigens and self-peptides, could explain why there may be a break in self-tolerance after infection with certain pathogens [50,51]. The idea underlying this mechanism is that self-peptides and bacterial antigens have homology that makes them cross-reactive, and thus CD8+ T cells would be primed to the 'foreign' antigen and then cross-react against self-peptides triggering pathogenesis. This hypothesis was strengthened in 1993 when HLA-B27-restricted cytotoxic CD8+ T cells (CTLs) from the synovial fluid of AS patients were found to recognize both bacterially infected and uninfected target cells [52]. This was evidence for the "arthritogenic peptide" model, where CD8+ T cells restricted to HLA-B27 need to be isolated from the arthritic joints of patients positive for SpA. Also, high homology was shown between a self-antigen derived from HLA-B27 itself and presented by this class I molecule (aa 309-320) and a peptide derived from *Chlamydia trachomatis* [53]. Later studies confirmed significant homology between self-peptides and peptides derived from members of the Gram-negative Enterobacteriaceae family, including *Klebsiella* [54], *Yersinia* [55] and *Salmonella* [56-58], that are presented by HLA-B27 [59]. It is unclear whether some of these sequences are generated *in vivo* or if HLA-B27 is able to present them directly. The fact that the DNA primase peptide (211-221) was endogenously processed (from its bacterial protein) and presented by HLA-B27 shows that this peptide might be the trigger facilitating the molecular mimicry between *Chlamydia* and the homologous HLA-B27 self-ligand (55% homology) and thus, associating HLA-B27 to disease [56]. The observation that infection with such bacteria

often precedes the onset of AS further supports this hypothesis as well [45]. In summary, these studies show how there could be molecular mimicry between microbes and self-antigens that could underlie triggering of AS.

Sequence similarities between human self-peptides presented on HLA-B*2705 and peptides derived from the Hepatitis B virus (HBV) suggest that molecular mimicry may also play a role in viral infections [60]. The similarities between HLA-B27 peptides derived from cartilage/ bone proteins and short peptide sequences derived from viruses known to cause chronic infections [59,21], support this. Since, AS is an inflammatory autoimmune disease primarily of the joints, it makes sense that the molecular mimicry between these 'self' and viral proteins could trigger disease. Another piece of supportive evidence is the extreme prevalence of the HBV surface antigen (HBsAg) in HLA-B27+ patients with AS, compared to other SpA patients, HLA-B27– AS patients and general population. This may indicate that the high prevalence of this antigen in AS patients might be associated with the expression of the HLA-B27 gene and the pathogenesis of the disease through molecular mimicry [61,62]. To reinforce this idea, Sun et al., also assessed the binding affinity between these viral peptides and HLA-B*2705 by SYFPEITHI epitope prediction database and Net MHC 3.4 server. This way, the sequences which do not bind HLA-B*2705 could be distinguished from the HLA-B*2705 candidate epitopes. In this study, it was predicted that among others, HLA-B*2705 can bind an HBV epitope which has molecular mimicry with human collagen. Also, crystallography data revealed that HLA-B*2705 can present the viral peptide pLMP2 (RRRWRRLTV), derived from the latent membrane protein 2 (residues 23-244) of Epstein-Barr virus (EBV) [63]. This indicates that the concept of molecular mimicry is not limited between bacterial peptides and self-peptides but also includes viral peptides.

However, the very few shared peptides (3% of the repertoire) by HLA-B27 and HLA-B14 [64] present a problem in defining the anchor residues of the arthritogenic peptide(s) [65]. This, along with the evidence that HLA-B27 disease in transgenic rats [66] does not require CD8+ T cells, makes it difficult to conclude that AS pathogenesis would be solely a consequence of cross-reactive CD8+ T cell responses between "self" and bacterial or viral mimic peptides. Briefly, another piece of evidence supporting this conclusion is that the cytokine IL-23 has been recently shown as a key factor in SpA. The misfolding of HLA-B27 triggers cellular stress response, followed by the production of IL-23 [67]. CD3+ CD4- CD8- T cells residing at the tendon-bone attachments (entheses) have been found to respond to IL-23 through their IL-23 receptor, thus producing the IL-6 IL-7, IL-22 and chemokine (C-X-C motif) ligand 1 (CXCL1), inflammatory mediators. Upon IL-22 production, the signal transducer and activator of transcription 3 (STAT3) gets activated and mediates inflammation at the entheses [68].

HLA-B27 and Misfolding

HLA-B27 has a unique peptide binding specificity which favors the theory that this class I molecule has the ability to present arthritogenic peptides. However, the lack of evidence supporting the arthritogenic peptide model *in vivo* has led to other hypotheses that could explain HLA-B27 and AS association. HLA-B27 must fold properly in the endoplasmic reticulum (ER) and associate with B2m and an antigenic peptide in order for it to be expressed on the cell surface, and therefore present the antigen to the CD8+ T cells. However, HLA-B27 also has an aberrant behavior [69]. Compared to other HLA molecules, it exhibits a slower folding rate and tends to misfold in the ER [70,71], leading to both stress in the ER and the activation of the unfolded protein response (UPR) [72]. UPR activates NF-κB and pro-inflammatory cytokines such as TNFα, IL-6 [73], and IL-23 [74] increase their expression. The resultant IL-23 can then stimulate a T-helper 17 cell (Th17) response, which may contribute to the pathogenesis of AS [75].

Because of these unusual HLA-B27 biological properties, the misfolding hypothesis was proposed [70].

A portion of assembled HLA-B27 heavy chains (HC) were shown to misfold because of the HLA-B27 B pocket [70] resulting in ER-associated degradation (ERAD) [76]. When the B pocket of HLA-B27 was replaced by the B pocket from HLA-A2, B27 HC could fold back [70]. Mear et al. [70] also compared the peptide-binding and peptide-loading features of both allotypes. The B27 misfolded HC were degraded in the cytosol, and overall less HLA-B27 molecules were loaded with peptide. Also, in the animal models misfolding is exacerbated: in mice due to the absence of endogenous B2m, and in rats by over expression of misfolded forms [66]. However, the study of a HLA-B27 transgenic rat model challenged the misfolding hypothesis [77], showing that an increased B2m expression could rescue the proper folding of the B27 HC. A reassessment of these results was done later on, where the HLA-B27 HC up-regulation was examined [67]. This study showed that extra B2m merely attenuates UPR activation, but it does not prevent it. Additionally, HLA-B*2707, which is usually, but not always, associated with AS, [78,79] has similar folding properties as the non-associated AS subtypes [80]. These properties were studied in terms of folding efficiency and export rate from the ER to the cell surface, measured by the acquisition of Endoglycosidase H (EndoH) resistance. Therefore, given the controversy of the results and the lack of correlation between the folding properties of AS-associated and non-associated subtypes, the evidence suggests that the misfolding hypothesis is probably not enough by itself to trigger the disease. Therefore, there must be some other important molecules (e.g., ERAP1 or tapasin, discussed below) and other HLA-B27 intrinsic properties influencing the development of AS and/or orchestrating the "right or wrong" behavior of the HLA-B27, which leads to its association with AS.

Oligomerization and intracellular accumulation patterns have shown a correlation between biochemical behavior and level of the predisposition to AS conferred by the different HLA-B27 subtypes [81]. This study demonstrated that along with an increase in their expression levels, AS-associated subtypes tend to accumulate in intracellular vesicles and form more oligomers than the non-associated subtypes. This is the only study so far showing a complete correlation between subtypes and AS, although the biological significance is still unknown. The authors argue that because all subtypes carry cysteine-67 (Cys67) [82] and other Cys residues important for homodimerization of B27, other factors may contribute to the association between the formation of oligomers in the associated subtypes and AS.

HLA-B27 and Cell Surface Homodimers

The canonical form of HLA-B27 at the cell surface is a heterodimer (HC-B2m) bound to a peptide. HLA-B27 can form polymers and covalent homodimers in the ER through the cysteine-67 (Cys67) residue in the α1 domain, as well as through other Cys residues [82,71]. Also, homodimers through just Cys67 can form at the cell surface. These structures are empty MHC-I molecules and arise by cell surface dissociation of heterodimers from B2m [83] or – possibly primarily – after dissociation from B2m after endosomal recycling [84]. The HLA-B27 homodimers hypothesis arose from this HLA-B27 ability to form homodimers [85], which offers another explanation for the association of HLA-B27 with AS. Briefly, even though HLA-B27 homodimers may not acquire appropriate peptides for cognate interactions with the T-cell receptor (TCR), the killer-cell Ig -like receptors (KIR3DL2) expressed on natural killer cells and CD4 Th17 cells [86] are able to recognize them. This recognition stimulates IL-17 production, which seems to be a link between the homodimers and the pathogenesis of AS as it triggers joint inflammation. More importantly, it has been shown that IL-17 production was increased in the blood and synovial fluid of patients with SpA, after KIR3DL2 (+) CD4 T cells

expansion and enrichment [75], further supporting the suggested link between this homodimers formation and AS. However, by itself this hypothesis does not completely explain the association between HLA-B27 and AS either. All the subtypes have the same cell surface homodimers formation potential, both their HC dissociated from B2m and free HC expressed at the cell surface were similar [87]. This is not surprising, since all the subtypes have a Cys residue at position 67.

Other Allotypes and Non-MHC Factors in AS

HLA-B14

As already stated in the introduction, the strongest association between a HLA class I molecule and any disease is the association of HLA-B27 with AS, but there have been other HLA class I molecules linked to AS as well. HLA-B27 has a strong preference for peptides containing arginine at P2 accommodated in the pocket B [28]. As mentioned above, this pocket B confers to HLA-B27 unusual unfolding properties that have been linked to the disease. This cavity is polymorphic among all the class I antigens and very few allotypes bind arginine at P2 in their peptides, making these allotypes more interesting in regards to their link to SpAs and AS. One of these allotypes is HLA-B14 which binds preferentially to peptides with arginine at P2 [88,64]. Additional evidence in support of the importance of the arginine at P2 is that the onset of SpAs in gorillas has been correlated with class I molecules which present peptides with arginine at P2 [89].

The HLA-B*1403 allotype is only found in the populations of Cameroon and Togo in Africa, where the prevalence of HLA-B27 is rare and the disease is infrequent, and it was found to be associated with AS [90,9]. Lopez-Larrea et al. [90] found in the study that in a small cohort of eight AS patients, four carried B*1403 and one carried B*2705, while 85 healthy controls (used to match for ethnic background) were found to be B*1402 positive. Given the fact that the size of the cohort used in this study was small, HLA-B27 is still the statistically strongest MHC-I associated with AS. B*1402 only differs from B*1403 in position 156: it is widespread among the Caucasian population and it has never been found to be associated to AS. These two HLA-B14 molecules, which are structurally similar but differentially associated to AS (Table 1), have been investigated as a way of testing the aforementioned hypotheses in a non-HLA-B27 system.

The peptide pool comparisons of the two HLA-B14 subtypes and that of HLA-B*2705 revealed that the two AS-associated allotypes, B*1403 and B*2705, share 3% of their peptide repertoires [64]. If the susceptibility to AS is based on the specific peptide recognition by T cells as is proposed by the arthritogenic peptide hypothesis [50], it would be expected to find common peptides with the same structural features between the two associated subtypes, B*1403 and B*2705. However, both the large disparity of their peptide repertoires and the lack of binding features shared by these two allotypes, but not B*1402, argue against (although do not exclude) a mechanism of spondyloarthritis by specific ligands of B*2705 and B*1403. The joint finding of a few shared ligands and cross-reactive CTL clones between HLA-B27 and HLA-B14 [64] suggests that B*1403 and B*2705 present either some shared peptides with the same antigenic features or distinct peptides showing antigenic mimicry.

A study comparing stability, maturation, assembly and folding properties of HLA-B*1402 and B*1403 to those of B*2705 [91], revealed that B*1402 and B*1403 have similar folding rates, faster and more efficient than B*2705. However, some unfolded HC from both B14 subtypes remained in the ER with a longer half-life than B*2705, indicating that their export rates are slower than B*2705. The finding of some Endo-H resistant HC for both B14 subtypes indicates that the heterodimers partially dissociate after exiting the ER. Thermostability and interaction with tapasin (a chaperone which brings peptides to MHC-I molecules)

was highest for B*2705 and lowest for B*1403. Altogether, this suggests that the B*1402-bound peptides and especially the B*1403-bound peptides were less optimized than those of B*2705. Because the biological features of B*1403 differ more from B*2705 than from B*1402, it does not seem that obvious that the underlying association with AS could be driven by the same biological properties in different associated class I allotypes. Therefore, it is worth, reassessing the significance of B*1403 and B*2705 sharing a low level of peptides and T cell epitopes. An obvious alternative, which does not oppose to the comparative biology of the three allotypes, might be a shared ligand of these two associated allotypes. However, in the absence of a formal demonstration of this shared arthritogenic peptide and the incomplete explanation of the association with AS, by the other hypotheses, other non-MHC factors have to be evaluated.

Other Components of the MHC-I Pathway

MHC class I pathway

Endogenous proteins are primarily degraded by the proteasome [92] which generates mature MHC-I epitopes usually between 8-11 amino acids long, depending on the class I molecule. In the case of HLA-B27, crystallography studies revealed nonamers as the most common bound peptides [93]. Approximately 10-15% of peptides are too long to bind directly to MHC-class I [94-96] and must undergo subsequent N-terminal trimming in the cytosol and/or ER. The peptides are translocated from the cytosol to the ER through the transporter associated with antigen processing (TAP) [97]. The endoplasmic reticulum aminopeptidase 1 (ERAP1) is the main responsible protease of N-terminal trimming of antigenic precursor peptides in the ER [98-100]. Endoplasmic reticulum aminopeptidase 2 (ERAP2) is also capable of this N-terminal trimming [101]. Following its proper folding, MHC-class I binds to B2m and is incorporated into the peptide-loading complex (PLC) [102]. The PLC, which consists of TAP, tapasin, calreticulin, calnexin, and ERp57, helps load MHC class I molecules with their peptide cargo [103].

ERAP1

Although HLA-B27 remains a dominant risk factor in susceptibility to AS, non-MHC molecules and other factors have been linked to the susceptibility of the disease [104]. In the last few years, these other factors are attracting more attention and more research has been conducted to better understand how the susceptibility to SpAs -and in particular to AS- is driven. ERAP1 was estimated to be the strongest non-MHC gene associated with AS, contributing to the association to the disease with a risk of 26% [105].

Different AS-associated ERAP1 single nucleotide polymorphism (SNPs) have been reported [106,107]. These different natural variants of ERAP1 have revealed different peptide length preferences as well as changes in the enzymatic activity [108] and stability of HLA-B*2704-peptide complexes [109]. García-Medel and colleagues showed that there is a correlation between ERAP1 polymorphisms associated with AS susceptibility, a efficient peptide trimming by this protease and high stability of HLA-B*2705, whereas protective polymorphisms against AS were associated to an attenuated activity of ERAP1, less active trimming, and decreased molecular stability of the class I molecule, suggesting less optimized HLA -B27 peptidomes. These findings suggest that the way in which ERAP1 and HLA-B27 interact is important in AS, and were consistent with those from a previous study where the SNP rs30187 (K528R) is a protective variant associated with reduced enzyme activity in vitro [110]. The SNP K528R, which is away from the enzyme's active site, controls the enzyme open-closed conformations, leading to more closed conformers which are consistent with decreased enzymatic activity [108]. On the contrary, the natural ERAP1 polymorphism predisposing to AS: R528K altered the expression levels of many HLA-B*2705-bound

peptides accounting for the association of this SNP with AS [111]. Currently, the main role of ERAP1 in MHC-I-associated AS seems to be through its effects on the MHC-bound peptidome [112]. However, due to ERAP1 involvement in angiogenesis [113] and macrophage activation [114], the existence of other inflammatory and immune pathways linked to AS through and indirect effect or ERAP1 cannot be ruled out.

Dendritic cells isolated from HLA-B27 AS patients expressed more ERAP1 than those from healthy individuals [115]. This finding was of interest since the SNPs identified in patients with AS by Harvey et al. [107] localized upstream the gene in a regulatory region, possibly impacting ERAP1 expression levels. Similarly, in a more recent study, the antigen presenting cells had their levels of gene expression affected by the SpA-associated ERAP1 polymorphisms. In dendritic cells and lymphoblastoid B cells isolated derived from these SpA patients, there was an association between ERAP1 SNPs predisposing to disease and higher ERAP1 mRNA expression levels, as well as higher ERAP1 transcripts or protein levels [116].

All these data point out that there is a correlation between: ERAP1 SNPs and predisposition to AS and higher ERAP1 expression both at mRNA levels and protein levels, as well as a more active enzyme. Since this protease plays an important role in the antigen processing and presentation in the MHC-I pathway, it is a critical link between susceptibility to AS and generation of peptide antigens to be presented by the MHC class I molecule HLA-B27.

Tapasin

Tapasin is a chaperone which binds HLA class I molecules [117], brings other members of the PLC onto TAP [118], shapes the HLA class I repertoire [119,220], increases the stability of HLA class I molecules [121-123] and influences both quantitatively and qualitatively the peptide repertoire [124].

The interactions between tapasin and HLA-B27 are mediated by the amino acids at positions 114, 116, and 152 in the peptide-binding groove of HLA-B27. These positions are key for these two molecules to interact with each other [125]. Interestingly, the B27 subtypes associated and non-associated with AS, differ at some of these positions. B*2705 and B*2709 are only different at the amino acid position 116, D116H [29]. This amino acid is located at the bottom of the F pocket, binding the C-terminus of the peptide [126]. B*2704 and B*2706 are different at positions 114 and 116: H114D and D116Y [30-32]. B*2707, an associated subtype, lacks D in position 116 (where a Y lies instead) like B*2706 and B*2709 which are not associated with AS. B*1402 and B*1403 differ only in position 156, where B*1402 has leucine and B*1403 arginine. This position has been suggested to affect the interaction between TAP and MHC-I [127], which is mediated by tapasin [117]. Experimental and theoretical research has proposed that the F pocket is the binding region of tapasin [128-131]. Since this pocket accommodates the C-terminal residue of the peptide bound to class I, one of the anchor residues, this suggests that changes in the interaction between tapasin and MHC-I could somehow drive the susceptibility to AS.

Some studies (discussed in the following paragraphs) have looked at how polymorphism changes in the B27 and B14 subtypes (already reviewed in the HLA-B14 section) may influence their interactions with tapasin, their dependency on tapasin to present peptides and what would be the consequences for AS susceptibility.

The tapasin dependence of a particular class I allotype was predicted using combinations of in silico and experimental approaches. These approaches used the sequence and crystal structure of a particular class I molecule. These approaches have demonstrated that B*2705 is more dependent on the chaperone than the conformationally stable B*2709

[132], in order to remain structured or properly folded and to bind peptides. A more unstable class I molecule would be more prone to misfolding and aggregation, thus being more susceptible to trigger pathogenesis. However, there is some controversy as to whether all the associated-subtypes have greater tapasin dependence than the non-associated subtypes. Some studies have analyzed B*2705-peptides complexes at the cell surface of tapasin-deficient cells and found that expression of B*2705 is independent of tapasin [124,121,133]. Compared to B*2705, B*2704 (another AS-associated subtype) is relatively dependent of tapasin for its surface expression [134]. However, in terms of maturation, these two associated molecules showed a similar tapasin dependency in this study. Both subtypes showed an inherent tendency to misfold, when tapasin is not present and too accumulated in the ER with relatively slow export to the cell surface. In contrast, B*2706 showed no accumulation in the ER and faster folding in the absence of tapasin. These results link tapasin to the misfolding hypothesis discussed above, as a potential explanation for the susceptibility to AS. It is worth noting though that B*2709, not associated to AS, matures similarly to B*2704 and B*2705, at least in the presence of tapasin [125]. In a different study, B*2709 was found to mature differently from B*2704 and B*2705 [80]. These two studies contradict each other and part of the reason for the different results could be that different cell lines were used to carry out these experiments. Again, the controversy and an imperfect correlation between the non-associated and associated subtypes with tapasin, do not explain the totality of the predisposition to the disease.

Microbiota and AS

The human microbiota, which represents the totality of microorganisms residing in the human body, has been recently presented as another factor in the etiopathogenesis of SpA. HLA-B27 and altered cecal microbiota have been associated [135]. The number of bacterial cells is 10-fold greater than human cells, being up to 100 trillion cells in the gut [136]. These organisms have been implicated in different aspects of the gut: maintaining homeostasis in a healthy state [137], regulating energy supply, controlling colonic pH, preventing the invasion of pathogens, and keeping intestinal health [138,139]. Bacterial dysbiosis promotes inflammation and may confer the development of human disease, linking bacterial composition and the immune system [140]. The intestinal microbiome in healthy individuals is now available thanks to the 16S ribosomal RNA (16S rRNA) sequencing technology. Nine divisions of bacteria comprise the microbiome and the majority of the species belong to four of them: *Bacteriodetes, Firmicutes, Proteobacteria, and Actinobacteria* [141]. Distinct clusters or "enterotypes" of bacteria that differ in their composition and function can also compose the human gut microbiome. The genus *Bacteriodetes* dominates the enterotype 1; *Prevotell*a, the enterotype 2 and, the enterotype 3 is dominated by *Ruminococcus* [142]. Species like *Prevotella* have been found to be increased in HLA-B27 transgenic rats and some other are decreased compared to wild-type rats [143].

The role of endogenous flora in the pathogenesis of AS has gained more relevance over the years, and increasing evidence supports the idea that there is a link between bacterial dysbiosis, HLA-B27, and AS. Inflammatory bowel disease (IBD) and AS have considerable clinical overlap and there is also an understanding that bowel flora play a role in IBD [144]. Around 7% of patients with AS have IBD, and 50-60% of AS patients have subclinical gut inflammation [145]. There are also some reports relating SpA and bacterial flora. *Chlamydia* for example triggers ReA, within the group of SpA, by inducing the expression of interleukin 23 (IL-23) in infected cells [146]. Several studies have reported distinct microbial colonization between AS patients and healthy controls, reviewed elsewhere [144]. Also, HLA-B27 has been proposed to alter the gut microbiome and to be linked to the development and severity of ReA. These patients are inefficient at eliminating the causative bacteria

[147]. Human monocyte cells where HLA-B27 was expressed, showed more impairment to handle intracellular replication of *Salmonella*, suggesting that the shape of the intestinal microbiome may be influenced by intracellular effects of HLA-B27 [148]. Some class I heterodimers tend to dissociate, presumably in the endosomal compartment after exiting the ER. This class I heterodimers that are in the endosomes are likely ones that have left the cell surface. This was showed for B*1403 and B*1402 where the percentage of Endo-H resistant free HCs increased within the time, along with a decrease in the percentage of HLA-B14 heterodimers [91]. This was interpreted as B14 having a less optimized repertoire of bound peptides, resulting in lower peptide stability. Indeed, B*1403 showed less binding of HC to tapasin over the time and less thermostability for its MHC/peptide complexes than B*1402. Most likely, following endosomal recycling, the B14-peptide complexes dissociate at late maturation stages as it has been described for B27 [84] and as a consequence of a less optimized repertoire of bound peptides. This dissociation could be triggered by the endosomal acidic pH, allowing peptide exchange in this compartment [149], where either endogenous or pathogenic peptides can be bound to class I molecules to be presented to CTLs. B*2705 did not show an increase in endo-H resistant free HCs [91], but their existence in HLA-B27+ AS patients cannot be ruled out. Thus, it could also be plausible that HLA-B27 is recycling to the phagolysosomes (a more acidic endosomal compartment) -where *Salmonella* resides- and because of its improper folding is unable to bind *Salmonella* peptides properly to present to CD8+ T cells, leading to a lack of immune response against this pathogen and therefore facilitating a persistent infection. The impaired elimination of microbes triggering ReA by HLA-B27+ monocytes may explain the persistence of these microbes in HLA-B27+ individuals susceptible to ReA [150,151]. Class I molecules can present exogenous antigens in a process called cross-presentation [152] and studies in mice have shown that dendritic cells, which are antigen professional presenting cells, can process *Salmonella* antigens and elicit *Salmonella*-specific CD8+ T cells responses [153]. In the case of HLA-B27+ AS patients, I postulate that this cross-presentation pathway could be defective and further research could facilitate the understanding of how the microbiota, HLA-B27 and probably other class I antigens, and AS are related.

In their hypothesis, Rosenbaum and Davey proposed that HLA-B27 shapes the human endogenous flora which causes AS [154]. However, a more recent study argues that immune dysfunction drives dysbiosis since immunological changes occur in the gut prior to any detectable microbial changes [155]. This does not rule out the possibility that HLA-B27 shapes the microbiome, but rather that immune dysfunction underlies these changes.

Penttinen et al. [148] also showed that glutamic acid at position 45 in the B pocket drives this reduced capacity to handle intracellular replication of *Salmonella*. This B pocket influences the folding properties of HLA-B27, which can lead to UPR, as already mentioned. However, Penttinen et al. [148] did not find evidence for an ongoing UPR. Along these lines, in a more recent study, data suggested that there is HLA-B27 misfolding in the gut of HLA-B27+ AS patients, together with autophagy activation rather than a UPR [156]. Autophagy and intestinal modulation of IL-23 in AS, appear to be associated. Also, AS patients with subclinical gut inflammation presented a local excessive production of IL-23 [157]. Autophagy is a process which helps in the maintenance of cellular homeostasis by degrading cellular constituents [158]. It is involved in host cellular defense against pathogens [159] and eliminates improperly folded proteins [160]. This targeting of improperly folded proteins for degradation occurs in the ER, similar to the UPR process [161] and it was suggested, that the inability to demonstrate UPR in all mentioned above studies could be due to compensation by excess autophagy [162].

HLA-B27 and TCR repertoire

Another theoretical mechanism for B27 association with AS is an altered TCR repertoire due to different positive or negative selection (and/or the development of regulatory T cells –Tregs-) on B27 in the thymus. Briefly, during positive selection in the thymus, only the thymocytes that interact appropriately (not too strongly or too weakly) with MHC-I molecules (also MHC-II) will receive a 'survival signal', thus the selected T-cells will have affinity to interact with MHC peptide complexes and to
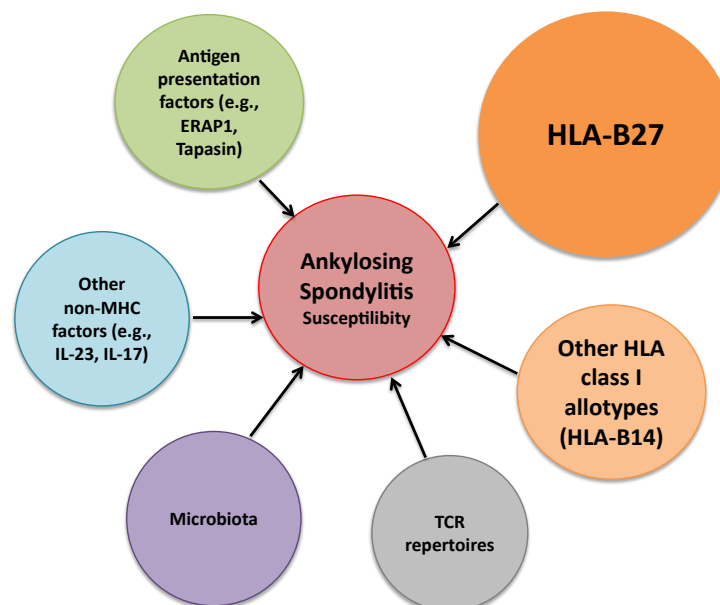
Figure 2: Contributions to AS susceptibility: Contributors to AS susceptibility include HLA-genes, non-HLA genes, antigen presentation factors, other non-MHC factors (not involved in the antigen presentation MHC-I pathway), the microbiota and the TCR repertoires. All these factors have taken HLA-B27 away from being the only risk factor to the susceptibility to AS. Thus, AS is a multi-factorial disease influenced by multiple molecules.

effect immune responses. Negative selection removes thymocytes capable of binding in a very strong way with 'self' MHC peptides, thereby self-tolerance can be maintained in order to avoid autoreactivity. It could also be that autoreactive T cells are redirected into Tregs cells [163]. One could speculate that if either one of these processes fail, it could lead to autoimmunity. If negative selection fails, then autoreactive T cells will not be eliminated, thus creating autoimmune disease. If in this case the selection is done on B27, autoreactive T cells would recognize self-peptides presented by the MHC-class I molecule, leading to AS. In the case of positive selection failure, T cells would not recognize foreign MHC-peptide complexes. The depletion of T cells would lead to a situation where a bacterial or viral infection would become persistent, triggering accumulation of complexes and MHC molecules. This accumulation of complexes leads to inflammation and AS, as discussed above (see HLA-B27 and misfolding section), even in the absence of T cells. Also if Tregs cells are not generated normally, then this could lead to autoimmunity. The contribution of altered TCR repertoires in the context of ReA and SpA was recently reviewed [164].

Conclusion

HLA-B27 is a peculiar MHC class I molecule with features that make it suitable to be linked with an autoimmune disease such as AS. It is a HLA class I molecule that binds and presents immunodominant peptides to cytotoxic T cells during important infections, such as; influenza, HIV, EBV, and hepatitis C [45]. As with all MHC molecules, B27 presents peptides but it also sets the perfect environment for T cell cross-reactivity, due to the high homology between self–peptides derived from the HLA-B27 molecule itself and microbial peptides. It tends to misfold in the ER producing stress through UPR, and the accumulation of free heavy chain allows for the formation of homodimers, at the cell surface. These features have been the main focus of the different hypotheses proposed to explain the link between HLA and AS. However, as evidenced by the comparative studies between different allotypes associated with AS (as e.g., HLA-B*2705 and HLA-B*1403) and between the different HLA-B27 subtypes; none of these hypotheses completely explain the association of these HLA-class I molecules with AS. The underlying mechanism of the association with AS seems more of a combination of the effects of many HLA, non-HLA genes (mainly covered in this review) and other factors, which within the last few years have gained more and more attention due to their shown link with AS. This together is taking HLA-B27 away from being the only risk factor to the susceptibility to AS (Figure 2).

Dissecting each of these hypotheses helps us to better understand the mechanisms underlying AS pathogenesis. However, because of the multiple molecules and mechanisms influencing the susceptibility to AS and the fact that all are involved in immune responses, it is worth considering that multiple of these mechanisms influence whether or not AS develops. Further study of these players would help us to elucidate the mystery behind the association of these components and HLA-B27 with AS, a disease that has been researched for the last 40 years.

Acknowledgment

Conflicts of Interest

None

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