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TITLE: HLA-B*27 and Spondyloarthritis: At the Crossroads of Innate and Adaptive Immunity

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Abstract

HLA-B*27 confers a strong risk for developing spondyloarthritis (SpA), which includes axial (AxSpA) ~~and/or~~ with or without peripheral arthritis, enthesitis, acute anterior uveitis (AAU) and gastrointestinal inflammation. While no definitive mechanism has been established to explain the role of this HLA class I protein in pathogenesis, three main hypotheses have emerged. First is the idea that self-peptides displayed by HLA-B*27 resemble microbe-derived peptides, leading to the expansion of autoreactive CD8+ T cells that trigger disease. The second two hypotheses focus on ~~Aberrant~~ aberrant properties of HLA-B*27 ~~discovered in the late 1990s~~, including its tendency to form cell surface dimers that can activate innate killer immunoglobulin-like receptors (KIRs) on CD4+ Th17 T cells triggering the production of pathogenic cytokines. ~~and to~~ HLA-B*27 also misfolds in the endoplasmic reticulum (ER), which can ~~can~~ activate the unfolded protein response, ~~. This~~ increasinges IL-23 expression thereby promoting the production of type 17 cytokines. ~~together with results from transgenic animal models, have suggested alternative mechanisms. For example, cell surface dimers and free HLA-B*27 heavy chains can activate innate killer immunoglobulin-like receptors (KIRs) on CD4+ Th17 T cells triggering the production of pathogenic cytokines. In addition, HLA-B*27 misfolding can activate the unfolded protein response. This increases IL-23 expression in myeloid cells which can promote the production of pathogenic type 17 cytokines.~~ HLA-B*27 misfolding in mesenchymal stem cells (MSCs) has also been linked to enhanced bone

formation by MSC-derived osteoblasts, which could contribute to structural damage in AxSpA. In this review we summarize prevailing ideas about the role of HLA-B*27 in SpA, discuss recent developments as well as limitations in our current knowledge, and provide recommendations for future research to address these unmet needs.

Introduction

Ankylosing spondylitis (AS) is the prototypic form of a family of diseases associated with HLA-B*27 and referred to as spondyloarthritis (SpA). SpA includes AS, reactive arthritis, ~~enteropathic~~ inflammatory bowel disease (IBD)-associated arthritis, juvenile arthritis (enthesitis-related arthritis), and psoriatic arthritis. Newer classification systems have defined axial SpA (AxSpA){Rudwaleit, 2009 #166}, which is increasingly used to describe AS and includes earlier stages of disease{Taurog, 2016 #159}. The strong association between HLA-B*27 and ankylosing spondylitis (AS) has been recognized since the mid-1970s{Brewerton, 1973 #160;Caffrey, 1973 #161;Schlosstein, 1973 #162}. HLA-B*27 is present in 85-90% of patients with AS, and when two copies are present (homozygosity) the risk of developing AS is increased [Jaakkola E et al., Ann Rheum Dis 2006, 65:775-780]. Heritability of AS has been estimated to be >90%. However, susceptibility genes identified to date account for only about 24% of heritability, with HLA-B*27 accounting for approximately ~~two-thirds~~ 80% of the known heritability ~~for this complex genetic disease~~{Brown, 2016 #169}. ~~However~~Nevertheless, HLA-B*27 is not sufficient to cause disease ~~since~~ and only approximately 5% of individuals carrying this allele develop SpA~~.~~ ~~Nevertheless.~~ However, the fact that the vast majority of individuals with AS carry HLA-B*27, and that overexpression of this allele in rodents can cause an inflammatory disease resembling SpA{Hammer, 1990 #179}, has positioned the HLA-B*27 heavy chain at the center of many studies aimed at

elucidating disease pathogenesis. The arthritogenic peptide hypothesis{Benjamin, 1990 #174} was stalled by a lack of progress in identifying CD8+ autoreactive T cells and the self-peptides they target, and then by the recognition that CD8+ T cells do not mediate disease in rodent models{May, 2003 #42;Taurog, 2009 #72}. In addition, the discovery of aberrant features of the HLA-B*27 heavy chain, including cell surface dimerization{Allen, 1999 #181} and its tendency to misfold during assembly in the endoplasmic reticulum (ER){Mear, 1999 #182}, suggested plausible alternatives to arthritogenic peptides. To date, the relative contribution of these mechanisms to SpA pathogenesis remains unclear, but has contributed to a robust discussion as to whether AxSpA is an autoimmune or autoinflammatory disease{Mauro, 2021 #165}. In this review we provide a brief historical perspective on the emergence of the three main hypotheses, and then focus on new developments that have rekindled interest in the search for arthritogenic self-peptides and implicated HLA-B*27 in aberrant bone formation. The clinical features and epidemiology of SpA, other susceptibility genes, and pathogenic mechanisms beyond HLA-B*27 are not the focus of this review.

The emergence of three main hypotheses about the role of HLA-B*27~~: arthritogenic peptides, innate immune receptor recognition of aberrant forms, and consequences of ER stress~~

Since 1990 three lines of evidence have emerged suggesting different roles for HLA-B*27 in disease. To understand the development of these concepts we provide a brief historical perspective which is shown in **Figure 1**. Whilst a key role of HLA class I molecules in the immune system was well appreciated by the 1980s, the field was revolutionized by the discovery that class I molecules present small protein fragments (peptides) coming from inside the cell following viral infection{Townsend, 1986 #170}. High resolution crystal structures of class I molecules revealed their antigen binding site as a peptide binding groove with self-peptides presented in the absence of viral infection{Bjorkman, 1987 #172;Garrett, 1989 #173}. These findings led to the arthritogenic peptide hypothesis, which broadly suggests that HLA-B*27-associated diseases result from “a (CD8+) T-cell-mediated anti-self-reaction directed at an as yet unknown peptide-HLA-B*27 combination”{Benjamin, 1990 #174} (**Figure 2**). If the presentation of a microbial-derived peptide triggered subsequent recognition of a self-peptide (or peptides), the CD8+ T cells would be autoreactive and cross-reactivity would constitute a true form of molecular mimicry.

Forced expression of HLA-B*27 along with human β_2-microglobulin (hβ_2m) in mice (HLA-B*27 Tg) was reported in 1987{Kievits, 1987 #178} and demonstrated that rodent T cells could recognize human leukocyte antigens (HLA). However, the mice did not develop inflammatory disease. However, adopting a similar approach in rats led to the discovery in 1990 that overexpression of HLA-B*27 and hβ_2m (but not HLA-B*7 and hβ_2m) can cause

gut inflammation and arthritis, two key features of spondyloarthritis{Hammer, 1990 #179;Taurog, 1999 #76}. Thus, in the early 1990s, armed with a novel hypothesis and an animal model, the field seemed poised to solve a nearly 20-year-old puzzle and define arthritogenic peptides presented by HLA-B*27 that could trigger disease.

In 1993 a study of individuals with reactive arthritis (a transient form of spondyloarthritis triggered by gastrointestinal and genitourinary infection) or AS demonstrated that patient-derived CD8+ T cells could recognize target cells infected with triggering organisms. Importantly, some of these CD8+ T cells also recognized uninfected cells, providing indirect evidence in support of arthritogenic peptides{Hermann, 1993 #180}. However, little additional evidence for autoreactive T cells in AS or other forms of SpA accrued over the next several years.

By 1999 it was apparent that HLA-B*27 exhibited aberrant properties{Allen, 1999 #181;Mear, 1999 #182}. Dimerized forms of HLA-B*27 were found on the cell surface{Allen, 1999 #181}, and newly-synthesized HLA-B*27 heavy chains were reported to misfold in the ER prior to the assembly of heavy chain–peptide–β_2m complexes{Mear, 1999 #182}. Previous studies had shown that HLA-B*27 has a very strong preference for peptides containing arginine at position-2 (P2) from the N-terminus{Jardetzky, 1991 #175;Madden, 1992 #176;Colbert, 1993 #177}. This is due to the combination of residues lining the “B” pocket of HLA-B*27, including a negatively charged glutamic acid at position 45 which interacts

with the positively charged peptide P2 arginine. There is also an unpaired cysteine at position 67 of the HLA-B*27 heavy chain{Colbert, 1993 #177}. Studies of misfolding and dimerization demonstrated that the B pocket, including this reactive cysteine residue, is responsible for the aberrant properties of HLA-B*27{Allen, 1999 #181;Mear, 1999 #182;Dangoria, 2002 #22}. Thus, the same structural features that link HLA-B*27 to the binding of potentially arthritogenic peptides are responsible for its aberrant properties.

The existence of cell surface HLA-B*27 heavy chain dimers and the tendency of the heavy chain to misfold opened up areas of investigation focusing on how this unusual allele might contribute to disease distinct from arthritogenic peptides. In parallel, studies emerging in the late 1990s and early 2000s in HLA-B*27 transgenic rats implicated CD4+ rather than CD8+ T cells in disease causation{Breban, 1996 #185;May, 2003 #42}, and fueled the fire for these alternative mechanisms. This was further supported by reports of arthritis in HLA-B*27 Tg mice lacking endogenous β_2m, which abrogates cell surface expression of HLA class I molecules displaying peptides and virtually eliminates the development of CD8+ T cells{Khare, 1995 #187}.

Expression of homodimers and even misfolded multimers of HLA-B*27 was found to be increased in cell lines with defects in their antigen processing and presentation apparatus. Aberrant cell surface complexes appeared to form primarily via endosomal recycling of cell surface HLA-B*27{Bird, 2003 #268} with misfolded HLA-B*27 heavy chains in the ER

prevented from accessing the cell surface (likely due to the cellular “quality control” mechanisms){Mear, 1999 #182;Dangoria, 2002 #22}. HLA-B*27-positive patients with AS also showed evidence of increased expression of non-conventional forms of HLA-B*27 on peripheral blood myeloid cells, in the joints, and in the gut{Raine, 2006 #190;Rysnik, 2016 #191}. It was subsequently shown that recombinant HLA-B*27 homodimers were able to bind to a number of innate immune receptors including killer immunoglobulin-like receptor (KIR) 3DL2 (KIR3DL2) and leukocyte immunoglobulin-like receptor (LILR) B2 (LILRB2) expressed on natural killer cells, subsets of CD4+ and CD8+ T cells and on B cells{Kollnberger, 2002 #188;Chan, 2005 #9}. Killer immunoglobulin-like receptors such as KIR3DL2 deliver primarily negative signals, but cell co-culture experiments showed that the interaction with aberrant HLA-B*27 appeared to deliver an anti-apoptotic signal resulting in expansion of KIR3DL2-positive CD4+ T cells which were also skewed towards enhanced production of the pro-inflammatory cytokine IL-17{Bowness, 2011 #193}. Together, these findings suggest that HLA-B*27 could trigger or promote disease via innate immune receptor recognition of aberrant cell surface forms of this allele.

It is also important to consider that other non-HLA-B*27 class I alleles have been shown to contribute risk (albeit much lower than HLA-B*27) to the development of AS{Cortes, 2015 #194;Hwang, 2021 #263}, and there is evidence for an epistatic interaction with HLA-B*60 where the presence of HLA-B*27 together with HLA-B*60 substantially increases disease risk [van

Gaalen F et al., Ann Rheum Dis 2013, 72:974-978]. . Whilst no unifying mechanism has been established, one possibility is that heavy chains encoded by other risk alleles can also form aberrant cell surface multimers dependent to a large extent on their position 97 amino acid residue{Cortes, 2015 #194}. This is supported by data using transfected cell lines{Chen, 2017 #195}. These intriguing findings require further verification but offer a potential link between several HLA-B alleles and pathogenic IL-17 production.

The propensity of HLA-B*27 to misfold led to the discovery that exposure of myeloid cells from HLA-B*27 Tg rats to cytokines that upregulate major histocompatibility complex (MHC) protein expression and components of the antigen processing and presentation pathway, led to the accumulation of HLA-B*27 dimers in the ER and activation of the unfolded protein response (UPR){Turner, #196;Turner, #197} (**Figure 4**). While ER dimers of HLA-B*27 can be degraded by quality control mechanisms such as ER-associated degradation{Mear, 1999 #182} and autophagy{Navid, 2018 #47}, inefficient ubiquitination of HLA-B*27 dimers appears to play a role in their accumulation{Navid, 2018 #47}, and might help explain why ER stress develops. The UPR activation is has several effects, including a homeostatic mechanism that reducesing the flux of proteins into the ER, and then expandsing the capacity of this organelle to fold, secrete, and/or degrade proteins{Navid, 2017 #199} (**Figure 4**). UPR activation can also lead to synergistic upregulation of certain cytokines such as IFNβ{Smith, 2008 #200} and IL-23{Navid, 2017 #199;DeLay, 2009 #23}. The key role of IL-23

in the development, survival, and activation of CD4+ Th17 T cells, together with earlier studies implicating CD4+ T cells in mediating the SpA-like disease in HLA-B*27 Tg rats, led to the discovery that Th17 cells were major components of inflammatory infiltrates in the gut{DeLay, 2009 #23} and joints{Glatigny, 2012 #202} of HLA-B*27 Tg rats. Whether or not there is a role for IFNβ in pathogenesis remains unknown. These observations suggested that HLA-B*27 misfolding-induced ER stress, UPR activation, and enhanced IL-23 production, might provide a link between this allele and the inflammatory phenotype of SpA{DeLay, 2009 #23} (**Figure 4**).

Recent evidence regarding the role of HLA-B*27~~developments~~

*HLA-B*27 and arthritogenic peptides*

Since the initial demonstration of *Salmonella*-reactive CD8+ T cells cross-reacting with uninfected cells in 1993{Hermann, 1993 #180}, evidence for autoreactivity and arthritogenic peptides has accrued slowly. Conserved T cell receptor (TCR) beta chain (TRBV) usage in cross-reactive CD8+ T cells from patients with ReA, provided stronger evidence in support of autoreactivity and identified specific TCR sequences that were expanded{Duchmann, 1996 #205;May, 2002 #206}. Other studies have shown that HLA-B*27 can present peptides from *Chlamydia*{Appel, 2004 #207}, however, the frequency of these CD8+ T cells was low and there was no evidence of pathogenicity or self-peptides as targets. Using an approach

based on candidate peptides an increased frequency of CD8+ T cell responses to a self-peptide derived from the vasoactive intestinal peptide receptor 1 (VIP1R) protein were described in AS patients{Fiorillo, 2000 #208}. This intriguing finding has not been widely replicated but deserves further attention.

Two key advances since 2017 have renewed interest in arthritogenic peptides and CD8+ T cells. First, the TRBV motif first described by in 2002{May, 2002 #206} has been confirmed in a much large number of patients with AxSpA using high throughput TCR sequencing{Faham, 2017 #163;Komech, 2018 #209;Komech, 2022 #210}. Most notably, the 2017 study included 234 AS patients and 227 healthy controls, including an HLA-B*27-positive group. They found that the TRBV9 motif was present at a higher frequency in AxSpA patients compared to both healthy control groups. These findings strongly suggest that TRBV9 T cells are enriched in AS over and above an effect of HLA-B*27 alone. The second major advance in 2022 analyzed the transcriptome of individual T cells from joint and uveal fluid of patients with AxSpA and acute anterior uveitis (AAU) using single cell RNA sequencing (scRNAseq){Yang, 2022 #164}. This study confirmed the increase in TRBV9 sequences including the conserved CDR3 Y/FSTDTQ motif, and identified T cell receptor α (TRA) sequences (e.g. TRAV21) that frequently pair with TRBV9 in the expanded CD8+ T cells. The authors then expressed TCR$\alpha\beta$ chains with the TRBV9/TRAV21 sequences found in AxSpA/AAU and used soluble TCRs to screen a library of peptides bound to

HLA-B*27. The library was produced in yeast, with each cell expressing HLA-B*27 covalently linked to β_2m and a peptide that differed in sequence between each cell. Using this approach, the investigators were able to identify self and microbial peptides that could be recognized by the (previously orphan) T cell receptors expanded in AxSpA and AAU. Several receptors recognize a single bacterial peptide (YeIH.232-240) which is expressed by both commensal *E. coli* and potentially pathogenic *Salmonella* and *Shigella sp.* Additional X-ray crystallography studies demonstrated that the conserved TRBV9-CDR3 and the TRAV21-CDR1 played important roles in specifically recognizing the self and bacterial peptides. A follow-up study identified an additional expanded ocular CD8+ T cell clonotype expressing TRBV5-5 in combination with the conserved beta chain CDR3 motif and paired with TRAV21, that recognizes YeIH232.240, indicating that TRBV9 is dispensible for recognition. ~~further characterized expanded CD8+ T cells found in ocular fluid and in peripheral blood of patients with AxSpA and AAU, and demonstrated there they can recognize a bacterial peptide (YeiH) expressed by commensal *E. coli* or potentially pathogenic *Salmonella* and *Shigella sp.*, and presented by HLA-B*27.~~ Interestingly, these CD8+ T cells expressed a mucosal gene set consistent with antigen encounter and differentiation in the gastrointestinal tract{Paley, 2024 #270}. It should also be noted that despite having similar antigen specificity, these ocular T cells expressed a TRBV5-4 or TRBV5-5 motif rather than TRBV9, indicating that TRBV9 is dispensable.

The authors hypothesized a key role for these cells in pathogenesis and suggested they may explain a link between the intestine and inflammation in the eye and joints they are expanded at these sites. However, this study did not demonstrate the function of these clones (e.g. ~~report~~ cytokine and/or cytotoxicity expression signatures), and thus additional studies will be necessary. Together these studies support a central tenet of the arthritogenic peptide hypothesis – that T cell receptors expressed by expanded T cells in SpA recognize HLA-B*27-peptide complexes. However, the nature, cell specificity, location, and identity of arthritogenic self-peptides remain to be determined.

HLA-B*27 and ER stress

While HLA-B*27 overexpression in rats clearly generates ER stress and can lead to UPR activation, evidence that UPR activation occurs when HLA-B*27 is expressed at physiological levels in humans is mixed{Navid, 2021 #211}. In early studies UPR activation was not found in peripheral blood derived macrophages even with HLA-B*27 upregulation{Smith, 2008 #212;Zeng, 2011 #107}, while UPR activation has been observed in these cell types by others{Feng, 2012 #24;Rezaiemanesh, 2017 #215}. One difficulty measuring UPR activation is that UPR target gene expression can be transient, and the threshold for activation may be cell-type dependent. In addition, it may be that instead of a complete UPR, single components of the pathway could contribute. This has been shown for missense mutations in

the TNF receptor (*TNFRSF1A*) that cause TNFR1 to accumulate intracellularly. This is associated with XBP1 splicing without upregulation of BiP and CHOP in cells from TNF receptor-associated periodic syndrome (TRAPS) patients{Navid, 2017 #199;Dickie, 2012 #216}. Cells from these patients also accumulate mitochondrial reactive oxygen species (ROS), which alters pro-inflammatory cytokine production and likely contributes to disease pathogenesis{Bulua, #217}.

The finding that UPR induction of C/EBP homologous protein (CHOP) mediates synergistic *IL23a* upregulation after triggering of toll like receptors (TLRs){Goodall, 2010 #27}, focused some attention on this UPR target gene in HLA-B*27-mediated disease. The role of CHOP in HLA-B*27-induced gut inflammation was explored using HLA-B*27 Tg rats{Navid, 2024 #218}. This study tested the hypothesis that eliminating CHOP would ameliorate gut inflammation if HLA-B*27 was driving increased IL-23 expression via UPR activation. However, CHOP-deficient HLA-B*27-expressing rats were found to exhibit slightly worse gut inflammation despite a reduction in *Il23a* expression. Interestingly, *Il17a* and *Il17f* were not substantially reduced despite the reduction in *Il23a*. Enhanced expression of cytokines such as *Tnf*, *Ifng*, *Il1a* and *Il1b* was found in the tissue in animals lacking CHOP, consistent with increased histology scores. These findings indicate that an HLA-B*27-induced UPR does not drive gut disease in this experimental model of SpA, but rather suggest a protective role for UPR-induced CHOP{Navid, 2024 #218}.

Interestingly, there is compelling evidence that HLA-B*27 expression could play a role in increased bone formation in AS patients through activation of the UPR transcription factor, XBP1{Liu, 2019 #219}. Mesenchymal stem cells (MSCs) isolated from spinal entheseal tissue obtained during surgery of HLA-B*27 positive AS patients exhibited enhanced mineralization when differentiated into osteoblasts compared to non-AS patient cells obtained from similar sites from trauma surgery patients. Bone formation pathway genes were not differentially expressed in AS cells during differentiation compared to controls, but rather there was increased expression of tissue non-specific alkaline phosphatase (TNAP; encoded by *ALPL*) in AS patient cells. TNAP can contribute to bone formation by increasing the pool of inorganic phosphate through pyrophosphate cleavage{Orimo, 2010 #220}. Inhibition of TNAP was sufficient to decrease the aberrant mineralization observed by AS patient-derived cells. Increased TNAP levels were detected in the serum of AS patients who exhibited more functional restriction and structural damage from their disease. Moreover, implants of the human MSCs into paraspinal areas of immunodeficient (NOD-SCID) mice revealed increased ectopic bone formation by AS patient-derived cells in a TNAP-dependent fashion. HLA-B*27 was both necessary and sufficient for increased TNAP expression as demonstrated by knockdown and overexpression experiments, and the effect was specific for HLA-B*27, establishing a direct role for this allele in aberrant mineralization. A link to ER stress activation was established by demonstrating misfolded forms of HLA-

B*27 in cells exhibiting IRE1 activation and increased XBP1 splicing, and that spliced XBP1 could upregulate the transcription factor retinoic acid receptor-β (RARB), which in turn upregulates TNAP expression{Liu, 2019 #219}. Interestingly, spliced XBP1 can also promote endochondral bone formation{Guo, 2014 #221}, although this mechanism was not investigated in this study. Whether spliced XBP1 promotes IFNβ production in MSCs, as has been shown in other cell types in the context of TLR stimulation, remains to be determined. IFNβ is a potent inhibitor of osteoclast formation and thus could contribute to a relative increase in bone formation independent of its effect on osteoblasts.

~~Other roles for HLA-B*27 in SpA~~

*Epistasis between HLA-B*27 and ERAP1*

There is a strong genetic interaction between HLA-B*27 and the ER aminopeptidase 1 (*ERAP1*) gene, with genetic variants in *ERAP1* conferring risk/protection mainly in HLA-B*27-positive cases of AS{Evans, 2011 #222}, while the effect also extends to HLA-B40 (B*40:01) in HLA-B*27-negative cases{Cortes, 2015 #194}. Although ERAP1 has more than one function{Tiburca, 2024 #223}, its prominent role in trimming peptides in the ER that are eventually presented by HLA class I proteins strongly implicates the quality and/or quantity of peptides available to HLA-B*27 in disease pathogenesis. Genetic and functional data indicate that ERAP1 variants that confer loss of function or reduced expression are associated with protection

from AS{Evans, 2011 #222;Reeves, 2013 #224;Costantino, 2015 #225}. Knocking down ERAP1 expression in cell lines to mimic reduced expression and/or loss of function clearly impacts the presence of various forms of HLA-B*27 as well as HLA-B*27-bound peptides, although the use of different cell lines and experimental conditions has led to variable results between studies{Haroon, 2012 #226;Zervoudi, 2013 #227;Chen, #228;Tran, #229}. A consistent finding has been that peptides presented by HLA-B*27 in the absence of ERAP1 are longer than the canonical octamers, nonamers and decamers found when ERAP1 expression is normal{Chen, 2016 #228;Barnea, 2017 #231}.

Epistasis between HLA-B*27 and ERAP1 has been modeled in rodents where eliminating ERAP1 expression protects HLA-B*27 Tg rats from developing arthritis without reducing gut inflammation{Tran, 2023 #230}. Since CD8+ T cell recognition of HLA-B*27 does not play a role in this model, a more important finding was that in the absence of ERAP1, HLA-B*27 folding was more efficient which reduced misfolding and mitigated activation of the UPR and the increase in *Il23a* expression{Tran, #230}. Thus, since peptide availability influences HLA-B*27 folding and cell surface stability, epistasis between HLA-B*27 and ERAP1 does not imply a predominant mechanism of pathogenesis.

It should be noted that epistatic interactions between ERAP1 and HLA class I are also seen with Behçet’s disease (HLA-B*51) and psoriasis (HLA-C) {Kirino, 2013 #232;Genetic Analysis of Psoriasis, 2010 #233}. While loss-of-

function variants of ERAP1 are associated with protection from AS, they create risk for Behçet's disease{Takeuchi, 2016 #235}, underscoring the complexity of these relationships.

HLA-B*27 and alterations in the gut microbiome

While there is considerable evidence from animal as well as human studies implicating gut microbial dysbiosis in the pathogenesis of spondyloarthritis, the role of HLA-B*27 is less clear. Pioneering studies done in the 1990s demonstrated that maintaining HLA-B*27 Tg animals in germ-free conditions could prevent the development of arthritis and gut inflammation{Taurog, 1994 #237}. It is also clear HLA-B*27 Tg rats develop gut dysbiosis{Lin, 2014 #236}, however, the characteristics of dysbiosis are highly dependent on the rat strain (i.e. genetic background) and/or environment, with no clear evidence for increases or decreases in specific bacteria across strains{Gill, 2018 #25}. Furthermore, it remains unclear whether changes in microbiota precede altered cytokine expression in the gut of HLA-B*27 Tg rats, or if the opposite is true. Separating out effects of HLA-B*27 in humans is even more complex due to heterogeneity among the other HLA alleles as well as dietary and environmental differences. Nevertheless, associations between HLA and microbial composition have been observed for HLA-B*27 as well as other HLA alleles{Asquith, 2019 #239}. Recent studies have emphasized a reduction in microbial diversity in spondyloarthritis patients compared to healthy controls, particularly in those who are HLA-B*27-positive, as well as overall

correlations between dysbiosis and disease activity with reproducible increases in *Ruminococcus gnavus*{Berland, 2023 #240;Breban, 2017 #241} which is known to produce a pro-inflammatory polysaccharide{Henke, 2019 #242}. The presence of HLA-B*27 could impact microbiota through any or all of the three mechanisms described above. Further studies are needed to better understand the role of microbiota in SpA.

HLA-B*27-ALK2 interactions

Ectopic expression of HLA-B*27 and β_2m in *Drosophila melanogaster* results in an abnormal phenotype characterized by loss of crossveins in the wings and in some cases reduced eye size{Grandon, 2019 #243}. This effect of HLA-B*27 was found to be due to an interaction of properly folded HLA-B*27/β_2m heterodimers with the type I bone morphogenic protein (BMP) receptor 1 saxophone (Sax). The HLA-B*27/β_2m interaction with Sax seems to disrupt Sax homodimerization, removing the antagonistic effect of Sax, thereby enhancing BMP signaling and preventing crossvein formation in the wings. The investigators went on to establish the relevance of this phenomenon in humans by showing that HLA-B*27 complexes exist on the cell surface in proximity to ALK2 (encoded by *ACVR1* in humans), which is the mammalian orthologue of the *Drosophila* Sax receptor. ALK2 is important for bone homeostasis, mediating BMP, TGF-β and activin signaling. They demonstrated that at baseline and upon stimulation with activin A or TGF-β, peripheral blood T cells from HLA-B*27+ patients exhibited increased

phosphorylation of the downstream mediators SMAD2and SMAD3 relative to healthy controls expressing other HLA class I proteins. This suggests that an HLA-B*27 interaction with ALK2 might prevent its inhibitory function on BMP signaling in SpA, possibly promoting the aberrant bone formation phenotype. This positive effect may be qualitatively similar to the effect of ALK2 (*ACVR1*) mutations that reduce binding to its natural inhibitor, resulting in the loss of antagonism and increased BMP signaling in fibrodysplasia ossificans progressiva. This disorder is characterized by progressive heterotopic ossification of connective tissue{Fukuda, 2009 #244}. Additionally, increased phosphorylation of SMAD2 and SMAD3 in HLA-B*27-expressing T lymphocytes might promote Th17 cell formation in SpA{Zhang, 2018 #245}. These important observations will need to be confirmed and extended to better understand the mechanism and consequences of this effect of HLA-B*27.

Implications for AS/AxSpA treatment

Landmark studies identifying TNF mRNA in sacroiliac joint biopsies from patients with AS provided a strong rationale for the use of biologics targeting this cytokine{Braun, 1995 #246}, which substantially advanced the treatment of AS in the early 2000s. The studies reviewed here implicating HLA-B*27 in activating the IL-23/IL-17 axis contributed to the development and validation of IL-17A inhibition in the treatment of AS/AxSpA{Baeten, 2015 #2}. It should be noted that type 17 cells produce

both IL-17A and IL-17F, and IL-17F may have pathogenic effects independent of IL-17A. To date only IL-17A inhibitors are approved for treatment of AxSpA. A recent study demonstrated efficacy of bimekuzimab, which blocks both IL-17A and IL-17F, in active AxSpA (Baraliakos X et al., Ann Rheum Dis 2024, 83:199-213). Additional studies will be needed to define the importance of IL-17F blockade. Interestingly, biologics targeting IL-23 (and IL-12/23) failed to show efficacy in AS/AxSpA{Baeten, 2018 #249;Deodhar, 2019 #250} despite strong genetic and pathophysiologic evidence that the IL-23 signaling pathway is important in this disease, and clear therapeutic efficacy in psoriasis{Reich, 2017 #251} and psoriatic arthritis{Fragoulis, 2022 #252}. The reasons for failure of IL-23 inhibition, particularly in the context of the success of IL-17A/F blockade, are not clear. They could include the possibility of an earlier therapeutic window that has closed in patients with AS/AxSpA studied in trials, and/or the importance of various innate immune cells that produce IL-17A/F independently of IL-23 stimulation, perhaps autonomously or through direct interaction with cell surface HLA-B*27 dimers that trigger NK or T cells{Baeten, 2020 #253}. JAK inhibitors (upadacitinib and tofacitinib) have also proven efficacious and are now approved for the treatment of AS/AxSpA{Hammitzsch, 2020 #254}, although the major cytokine pathways that are affected remain unclear. It will be interesting to learn whether TYK2 inhibitors (e.g. deucravactinib) are efficacious in AS/AxSpA as they have the potential to be better for blocking type 17 innate and adaptive immune cell dysfunction{Hromadova, 2021 #255}. The

success of deucravacitinib in a pre-clinical model of spondyloarthritis supports this idea{Gracey, 2020 #256}, but awaits confirmation.

The discovery and further characterization of T cells expressing HLA-B*27-restricted TRA21-TRBV9 and other TCR motifs in AS/AxSpA provides an opportunity to test new cell-based therapies in this disease. For example, chimeric antigen receptor (CAR) T cells directed at the conserved TCR chains might be used to selectively deplete T cells that are critical to disease initiation and/or progression. Conceptually, therapies based on displacing targeted self-peptides or even reducing HLA-B*27 expression could be considered. Intriguingly, there is a recent report of an AS patient with over 30 years of disease who was treated with an anti-TRBV9 monoclonal antibody which effectively depleted TRBV9-positive CD8+ T cells in the peripheral blood{Britanova, 2023 #257}. This was associated with substantial clinical improvement including measures of mobility and function that are associated with ankylosis and usually considered irreversible{Braun, 2024 #258}. The patient’s previous treatment was also highly unusual, having received an autologous hematopoietic stem cell transplant for AS a decade earlier. This is nevertheless a very promising result justifying further study. Early results from a randomized, double-blind, placebo-controlled trial of BCD-180 (anti-TRBV9) were recently reported, demonstrating that up to 50% of patients receiving BCD-180 achieved an ASAS40 response at week 24 compared to a similar ASAS40 response in only 24% of patients receiving placebo (Nasonov EL, 2024, Sci Pract Rheumatol 62:65, DOI:10.47360/1995-4484-2024-65-80).

The enthusiasm for anti-TRBV9 approaches must be tempered by the fact that there are TRBV5-4 and TRBV5-5 motifs recognizing the same antigen that could contribute to disease and yet not be targeted by anti-TRBV9 antibodies{Paley, 2024 #270}.

Future Perspectives

Research into the mechanisms by which HLA-B*27 contributes to the pathogenesis of SpA continues to advance, although defining the most proximal and causal events early in disease remains a significant challenge. Technological and conceptual advances in our understanding of innate and adaptive immunity and effects of HLA-B*27 expression have raised a raft of questions that should be of interest to the research community, some of which are listed in **Table 1**.

There is now direct evidence that CD8+ T cells bearing αβTCRs that are expanded in AS and AAU can recognize HLA-B*27-bound peptides{Yang, 2022 #164}, and some may develop after encountering bacterial antigens in the gastrointestinal tract providing a clue to their origin{Paley, 2024 #270}. This work has galvanized the field, but we need to learn more about the phenotype, function, and cytokine expression pattern of these T cells, as well as identifying self-peptides that they are targeting if they are indeed autoreactive as is anticipated. CD8+ T cells bearing these public TCRs have been found in joint and vitreous fluid of patients with AxSpA/AAU and would be expected in other sites of inflammation such as entheses. In addition to

public clones, private clones that are not broadly shared by patients have been discovered{Faham, 2017 #163}. Whether or not they are also HLA-B*27 restricted or if other HLA class I alleles present in HLA-B*27+ individuals are the target of additional autoreactive CD8+ T cells will be of interest. Such expansions could differ between individuals due to HLA heterogeneity and would have escaped detection to date. Broader autoimmunity could be the case if aberrant properties of HLA- B*27 work in concert as an innate immune stimulus to promote type 17 and/or CD8+ T cell autoreactivity. It is worth noting that carriage of HLA-B*27 is associated with better outcomes following infection with viruses such as hepatitis C and HIV{Neumann-Haefelin, 2010 #264;O'Brien, 2001 #265}. The basis for these associations is not known but could be related to oligomeric intracellular or extracellular forms of HLA-B*27 serving as innate immune stimuli. If there is a more general phenomenon of autoreactivity, the question of whether other genetic factors contribute to a loss of immune tolerance should be considered. These questions cannot be addressed using existing animal models where disease develops independently of CD8+ T cells, and thus studies of human cells and tissues or new models will be required.

Whilst the arthritogenic peptide hypothesis in its original form proposes that autoreactive T cells are cytotoxic (i.e. kill target cells) it is plausible that they may act primarily via production of inflammatory cytokines. Traditionally this would include TNF and IFNγ, although production of IL-17A/F

(i.e. Tc17 cells) might be expected in AxSpA. It will be important to perform detailed phenotyping and functional analysis of TRBV9-motif-bearing CD8+ T cells in AxSpA patients and controls in this context. Given the established importance of type 17 innate and adaptive lymphocytes and their cytokines in AxSpA/AS pathogenesis, establishing a link between expanded CD8+ T cells and Th17 cytokines is a high priority, as well as determining whether CD4+ T cells express this or other TCR$\alpha\beta$ motifs.

It would be surprising if the widespread tissue inflammation seen in AS/AxSpA (e.g. entheses, axial and peripheral joints, gastrointestinal tract, and eyes) will be explained by a single peptide targeted by autoreactive T cells, and thus looking for additional expanded TCR motifs and cross-reactive peptides is relevant. Characterization of expanded TCR motifs should extend to early disease, including juvenile-onset SpA, to establish the primacy of these cells and further specificity for HLA-B*27.

These new developments hold promise for novel biologic or cell-based therapies aimed at eliminating the expanded T cells. Early results are intriguing, but larger studies must be done to establish therapeutic efficacy and provide correlates with changes in imaging and/or tissue inflammation.

CD8+ T cells expressing inhibitory KIRs have recently been shown to efficiently eliminate pathogenic gliadin-specific CD4+ T cells from the leukocytes of celiac disease patients *in vitro*{Li, 2022 #259}. Considering the previous evidence supporting the regulation of CD4+ T and NK cells by HLA-B*27 via its binding to KIR3DL2{Bowness, 2015 #6}, it is worth

considering if KIR+ CD8+ T cells are relevant to AxSpA. More specially, KIR+ CD8+ T cells could have a regulatory role for CD4+ Th17 cell expansion. If this is the case, assessing whether the KIR+ CD8+ T cells in AxSpA are clonally expanded and restricted to HLA-B*27 would be of interest. Future work performing immunophenotyping and functional studies of KIR+ CD8+ T cells from blood or joint tissue will be important.

While ER stress and enhanced IL-23 production caused by HLA-B*27 expression in myeloid cells has been established in rats{DeLay, 2009 #23}, similar consequences have not been observed in humans. Moreover, a mechanistic link between UPR-induced CHOP/IL-23 has been ruled out as a potential mechanism for gut inflammation in rats{Navid, 2024 #218}. A recent study demonstrated that Th17 cell-inducing gut bacteria can induce ER stress in intestinal epithelial cells (IECs){Duan, #261} which could potentially be exacerbated by HLA-B*27 expression. Notably, activation of these IECs enhanced their production of both reactive oxygen species (ROS) and purine metabolites to promote Th17 cell accumulation, which are key drivers of inflammation in AxSpA. Thus, it would be of interest to determine whether HLA-B*27 enhances ER stress in IECs, which could alter Th17 generation and gut microbiota independent of the effects of CHOP on IL-23.

The discovery that HLA-B*27 can inhibit ALK2 function{Grandon, 2019 #243} has implications for altering signaling responses to TGF-β as well as BMPs, with potential downstream effects on immune homeostasis through regulation of T cell subsets (e.g. Th17, Treg, and Trm cells for TGF-β){Chen,

2023 #262} as well as bone formation. The molecular basis and consequences of this effect of HLA-B*27 require further study.

In addition to HLA-B*27, other HLA class I alleles (e.g. HLA-A*02:01, HLA-B*40:01, HLA-C*12:02) and some HLA class II alleles have been associated with an increased risk for developing AS in HLA-B*27-negative individuals{Hwang, 2021 #263}, although effect sizes for each allele are much smaller than that of HLA-B*27. Notably, while there is epitasis between ERAP1 and HLA-B*40:01 in conferring risk for AxSpA{Cortes, 2015 #194}, this has not been reported for other HLA class I alleles. The association of multiple HLA class I and II alleles with AS makes it unlikely that a single arthritogenic peptide exhibiting promiscuous binding to all these alleles is causative. While searching for expanded TCR clones in individuals with other risk alleles and/or assessing the biology of these risk alleles is attractive, it may be technically difficult since so few cases exist relative to HLA-B*27-positive patients.

Conclusions

William of Ockham, the noted 14th century English philosopher and theologian, noted that “Entities must not be multiplied beyond necessity”. What came to be known as Occam’s (or Ockham’s) razor implies that the simplest explanation is usually the best one. Unfortunately, the conundrum of HLA-B*27 and disease has so far defied simple explanations.

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References

Key points:

- The role of HLA-B*27 in the pathogenesis of axial SpA is incompletely understood.
- Evidence implicates canonical HLA-B*27-bound peptides as a target for CD8+ T cells, while aberrant forms of HLA-B*27 may engage KIRs on CD4+ Th17 T cells in disease.
- HLA-B*27 misfolding is implicated in activating mesenchymal stromal cells (MSCs) to promote mineralization by MSC-derived osteoblasts.
- ERAP1 loss-of-function, which reduce the risk of arthritis in HLA-B*27 positive individuals and animal models, alters the repertoire of peptides presented by HLA-B*27 as well as improving folding and reducing misfolding and ER stress.
- HLA-B*27 may also impact TGF-b and BMP signaling by inhibiting the function of ALK2.
- Canonical and non-canonical effects of HLA-B*27 suggest it may play more than one role in disease predisposition and place it at the crossroads of innate and adaptive immunity.

Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

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Table 1: Future perspectives for HLA-B*27-related mechanisms

Pathways related to HLA-B*27	Important questions	Potential research strategies
Clonally expanded	What is the phenotype of	Paired scRNA/TCR

CD8+ T cells and recognition of 'arthritogenic' peptides	TRAV21/ TRBV9+ CD8+ T cells and are they expanded in entheses, synovial tissue, and the gut? What is/are the autoantigenic (arthritogenic) peptide(s)? Are expanded CD8+ T cells with private TCRs HLA-B*27 restricted? Do CD4+ T cells express TRBV9 or other expanded sequences?	sequencing and spatial analysis of affected tissues Screening of tissue expression libraries using TCRs constructed from expanded clones Further analysis of expanded CD8+ T cells In-depth analysis of CD4+ T cell TCRs in AxSpA
Regulatory CD8+ killer T cells	Are KIR+ CD8+ T cells relevant to AxSpA? Are they regulated by aberrant HLA-B*27?	Immunophenotpying and functional analysis of KIR+ CD8+ T cells in AxSpA
Consequences of ER stress	What cells are affected by HLA-B*27-mediated ER stress? Are gut epithelial cells affected? Is UPR activation incomplete, with enhanced XBP1 splicing representing the key pathway?	Assess effects of HLA-B*27 expression in gut epithelial and other cell types Does activation of XBP1 splicing require additional stimuli in HLA-B*27 expressing cells?
ALK2 inhibition and enhanced TGF-b/BMP signaling	How are immune cells impacted by HLA-B*27 effects on the ALK2 pathway? Does this mechanism alter bone formation?	Biochemical and molecular analysis of the ALK2/HLA-B*27 interaction Analysis of immune cells and MSCs/osteoblasts

Figure Legends

Figure 1. Timeline depicting major events in research on the role of HLA-B*27 and spondyloarthritis since 1990. Events on top of the timeline (green shading) are related to autoimmunity where HLA-B*27 is targeted by adaptive immune cells, while events below the timeline relate to alternative hypotheses including HLA-B*27 serving as a target for innate immune receptors (KIRs), a trigger for cytokine production, or a stimulus for mineralization and new bone formation.

Figure 2. Schematic representation of the arthritogenic peptide hypothesis where HLA-B*27 presents self-peptides that resemble microbe-derived peptides and this become the target of cross-reactive CD8+ T cells. More details on T cells (TRBV9, origine of T cells etc)

Figure 3. Schematic representation of innate immune receptor (KIR3DL2) recognition of HLA-B*27 heavy chain (HC) dimers triggering IL-17A/F production from CD4+ T cells that have no T cell receptor specificity HLA-B*27.

Figure 4. Schematic representation of HLA-B*27 misfolding causing ER stress and triggering activation of the unfolded protein response (UPR). The UPR can promote IL-23 production from myeloid cells and *ALPL* (TNAP) upregulation in MSCs, thus promoting Th17 development and activation, as well as mineralization of MSC-derived osteoblasts. (More details about UPR)