## EDITORIAL

## HLA-B27: The Story Continues to Unfold

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The strong association between HLA-B27 and spondyloarthritis, in particular ankylosing spondylitis (AS), was first recognized over forty years ago. Since then a series of hypotheses, based initially on the canonical function of HLA-B27 and more recently on its aberrant behavior, have been proposed to explain its role in disease. While progress has been made, the precise role (or roles) of HLA-B27 in spondyloarthritis remains unclear (1). In the meantime, genome-wide association studies have implicated over 30 genes or genetic regions outside of the major histocompatibility complex (MHC) involved in susceptibility to AS (2). Together with animal models and translational studies, this has strongly implicated activation of the IL-23/IL-17 axis in disease pathogenesis (3). In addition, the identification of a unique population of entheseal-resident T cells in mice that are exquisitely sensitive to IL-23 may help to explain the spondyloarthritis phenotype (4). Thus, while tumor necrosis factor (TNF)-inhibitors are well-established options for control of active disease symptoms, there is a rationale basis to target additional cytokines and pathways (5, 6).

Teasing out the direct influence of HLA-B27 in disease has had a rather bumpy ride. The concept of 'arthritogenic' self peptides that become so by virtue of their resemblance to microbial peptides and trigger autoreactive CD8+ T cells, lacks significant experimental support. Moreover, there is clear evidence that CD8+ T cells are not required for the development of spondyloarthritis in HLA-B27 transgenic rats. Two concepts emerged from the discovery that HLA-B27 displays aberrant folding/unfolding properties compared with other HLA class I molecules (1, 7). To add complexity to the picture, there are at least two sites in cells where HLA-B27 deviates from the norm. First, in the endoplasmic reticulum (ER) newly made heavy chains are slow to fold, which results in the formation of disulfide-linked dimers. The accumulation of such misfolded proteins may generate ER stress and trigger an unfolded protein response (UPR) and ER-associated degradation (ERAD) pathways in an attempt to resolve the burden on the ER (8). Second, after HLA-B27 reaches the cell surface it tends to unfold resulting in the formation of free heavy chains. While cell-surface free heavy chains are not unique to HLA-B27, it has a propensity to form dimers during endosomal recycling resulting in the expression of aberrant cell surface complexes that can be recognized by leukocyte

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immunoglobulin-like receptors, most notably KIR3DL2. Interestingly, aberrant folding and unfolding of HLA-B27 have been linked to the IL-23/IL-17 axis through UPR-mediated increases in IL-23 production, and triggering of CD4+ Th17 T cells that express KIR3DL2, respectively. These concepts remain as attractive alternatives to the arthritogenic peptide hypothesis, but the extent to which they account for the role of HLA-B27 in spondyloarthritis remains to be determined. Furthermore, effects of HLA-B27 expression on dendritic cell function promote Th17 development in rats, although the molecular mechanism remains to be elucidated.

'B27' designates a family of HLA-B alleles called subtypes, with over 100 described to date (IMGT/HLA database; http://www.ebi.ac.uk/ipd/imgt/hla/align.html). Subtypes generally differ by 1 to 6 or 7 amino acids in the mature protein, while different B alleles might contain 15-30 differences (e.g. HLA-B27 and HLA-B7 differ by approximately 20 amino acids, or 6% of the protein). Most HLA-B27 subtypes are extremely rare, with many reported from single individuals, and thus their relationship with AS cannot be discerned. For some of the most commonly occurring subtypes the picture is somewhat clearer. For example, HLA-B*27:05 (B*27:05) and B*27:04 are strongly associated with AS, whereas B*27:06 is clearly not associated. The relatively rare HLA-B*27:09 allele is either not, or is only weakly associated with AS. These four subtypes differ by only 1-4 amino acids located at positions 77, 114, 116, and 152. Differences at amino acids 77 and 152 do not correlate with disease susceptibility, while differences at positions 114 and 116 located at the base of the peptide-binding groove seem to be more critical. The non-associated subtypes B*27:06 and B*27:09 have either Asp at 114 and Tyr at 116 (B*27:06), or His at 116 (B*27:09), while B*27:05 and B*27:04 both contain His at 114 and Asp at 116. Alterations in these residues can alter the types of peptides that bind, though there is significant overlap in the peptide repertoires of all 4 subtypes. Nevertheless, differential peptide binding has been used to support the notion of arthritogenic peptides, presuming a disease-causing peptide would be bound by B*27:04 and B*27:05 subtypes, but not by B*27:06 and B*27:09. Further refinement of this idea was prompted by elegant structural studies of B*27:05 and B*27:09 bound to a self-peptide derived from the vasoactive intestinal peptide type 1 receptor (sequence RRKWRRWHL) (pVIPR) (9). When bound by

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B*27:05, pVIPR was displayed in two different conformations with the central section of the peptide partially rotated, altering the potential antigenic surface presented to CD8+ T cells. Indeed, cytotoxic T lymphocytes could be generated *in vitro* that differentiated between the two orientations of the same peptide. In contrast, pVIPR bound to B*27:09 was observed in only one conformation, leading to speculation that a dual conformation peptide might generate autoreactivity.

In this issue of *Arthritis & Rheumatology*, Loll *et al*. have extended their analysis of HLA-B27 subtypes to include x-ray crystal structures of B*27:04 and B*27:06 with the pVIPR peptide. Rather than observing dual peptide conformations with the disease-associated B*27:04 subtype, this time it was observed for the ***non-disease-associated*** B*27:06. Thus the ability to present the same peptide in two conformations does not correlate with disease susceptibility as originally thought, nor does it require His 114 and Asp 116 in HLA-B27. The story might have ended here, but the authors followed up on their previous observation that B*27:05 was more flexible than B*27:09 (10). In the current study Loll *et al*. again employed a technique called infrared (IR) spectroscopy, but this time to probe the structures of B*27:04 and B*27:06 complexed with peptide and $\beta_2$-microglobulin ($\beta_2$m). IR spectroscopy examines dynamic properties of proteins in contrast to the static structures visualized by x-ray crystallography. The protein in solution is excited with IR energy and its response is examined. As with B*27:05 and B*27:09, there was a clear delineation between B*27:04 and B*27:06. Furthermore, as with the previous study, the disease associated subtype (B*27:04) exhibited a greater conformational flexibility than the non-disease associated allele (B*27:06). Increased conformational flexibility means a less rigid or more floppy structure. Intrinsically disordered proteins also exhibit greater flexibility with IR spectroscopy. Thus, rather than dual conformation peptides suggesting a subtype-specific basis for autoreactivity, the authors propose that greater structural flexibility or floppiness may contribute to disease. The question is how might this occur?

Increased conformational flexibility seems more consistent with models implicating HLA-B27 misfolding or unfolding in the pathogenesis of AS. Indeed, the authors argue that reduced

stability of the peptide-binding groove could support increased heavy chain dimerization and/or contribute to the development of ER stress. While the current studies are based on the biophysical properties of HLA class I complexes containing heavy chain, peptide, and $\beta_2$m, the results are strikingly reminiscent of experiments showing that newly-synthesized B*27:05 heavy chains are slow to fold and acquire a compact structure *in vivo*, which is associated with prolonged binding of ER chaperones and results in the formation of aberrant disulfide bonds (11-13). Furthermore, recent studies of Jeanty *et al*. showed increased oligomerization and intracellular accumulation of B*27:05 compared to B*27:06 when these heavy chains were transiently overexpressed in cells (14); precisely what one might predict if these two subtypes differ in their folding and/or unfolding properties. Of course conformational flexibility could also be hypothesized to lead to autoreactivity. The consequence of varied structure might be heterogeneous complexes that do not reach the density required for efficient negative selection of self-reactive CD8+ T cells. This would be consistent with B*27:05 and B*27:04 being disease associated, but not B*27:09. This leaves B*27:06 as the outlier since it is not disease associated but should lead to autoreactivity by virtue of dual conformation peptides. The notion that differential dual peptide conformations play a role for B*27:05/B*27:09, but conformational flexibility is the reason that B*27:04 is associated but not B*27:06 is difficult to reconcile.

As alluded to previously, the dichotomous separation of subtypes into disease-associated and non-disease associated may be too rigid for B*27:09. This subtype is relatively rare, discovered and mostly limited to the Sardinian population where it is part of an extended HLA haplotype. Thus, one caveat is that the statistical power to detect and quantify a weak association between B*27:09 and AS or undifferentiated spondyloarthritis, as suggested by numerous case reports is limited.

Ultimately, all HLA-B27 subtypes (and all HLA class I proteins) function as peptide-antigen presenting molecules. Indeed, it is only through the binding of peptides and $\beta_2$m that HLA class I heavy chains achieve the stability necessary to function. It then becomes relevant to ask

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whether the nature of the peptide pool – either quantitative or qualitative differences – might influence the conformational flexibility of HLA-B27 subtypes? It is noteworthy that variants in ER aminopeptidases (ERAP1 and ERAP2) have been implicated in susceptibility to AS (2, 15). ERAP1/2 are important proteases that trim peptides in the ER to optimize their length for binding to HLA class I proteins. Genetic and functional studies suggest that loss of ERAP1 function is protective, although more recent analyses of ERAP1 allotype pairs raise the possibility of a more complex relationship where either high or low activity could be detrimental (16). In any event, it would not be surprising if certain peptides were better at limiting the conformational flexibility of HLA-B27, thereby promoting its folding and limiting unfolding.

Detailed and careful structural analyses such as those provided by Loll *et al*. provide an important perspective on the biology of HLA-B27 variants that may prove to be relevant to disease pathogenesis. They emphasize the need for further studies probing structural differences, including dynamic processes such as folding and unfolding. Incorporating complex mixtures of peptides generated with varying influence of ERAP1/2 that better mimic the ER environment is a challenge that if met, may provide unprecedented insights into the role of HLA-B27 in spondyloarthritis.

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