Editorial

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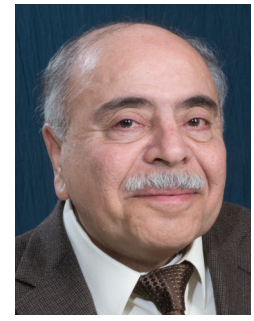
Gene–gene interactions of *HLA-B27* and *ERAP1* in ankylosing spondylitis: finding the pieces of the puzzle

"The epistasis between *HLA-B27* and *ERAP1*, the two genes that together provide the most powerful disease risk factors for ankylosing spondylitis, implicates aberrant peptide handling in the endoplasmic reticulum as a contributory factor."

Keywords: ankylosing spondylitis • endoplasmic reticulum stress response • epistasis • *ERAP1* • gene–gene interaction • *HLA-B27* • misfolding • pathogenesis • spondyloarthritis • unfolded protein response



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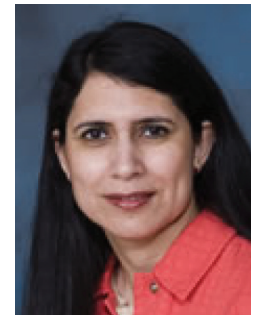

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Background

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease of unknown etiology with a strong genetic susceptibility. Significant progress is being made in our understanding of the pathogenetic mechanisms involved in this disease, and the recent genome-wide association study (GWAS) results have linked at least 60 loci to AS [1]. Although GWAS has yielded remarkable results in associating different genetic polymorphism to AS susceptibility, many of these candidate genes have not been well characterized. However, these studies have revealed some important biologic pathways that may be involved in etiopathogenesis of AS [1,2].

HLA-B27 and *ERAP1* are the first two genes that have been found to increase the risk for AS [1,3]. The relative attributable risk of *ERAP1* to AS is approximately 25%, and that of *HLA-B27* is approximately 50%. So these two genes combined provide the two most powerful disease risk factors to AS. We are going to restrict our commentary to focus only on the possible genetic interactions between *HLA-B27* and *ERAP1* and their role in the pathogenesis of AS.

Genes do not work independently but in concert, much like in an orchestra. In a recently published study of patients with schizophrenia, the research team found that only after organizing the genetic variations and the patients' symptoms into categories were they able to recognize that particular clusters of DNA variations act together to cause specific types of symptoms [4]. This new research shows that schizophrenia is not a single disease but a group of eight genetically distinct disorders, each with its own set of symptoms [4]. A similar approach is needed to better understand how genes work together to cause some of the other relatively common but complex and genetically heterogeneous disorders, including AS.

Role of HLA-B27 in AS

The remarkable association of *HLA-B27* with development of AS and related forms of arthritis grouped under the term spondyloarthritis (SpA) was first reported in early 1970s, and there is a mounting evidence indicating a direct role of *HLA-B27* in disease pathogenesis [1,5,6]. This is further supported by the spontaneous development of SpA-like disease in *HLA-B27*-transgenic rats [7]. However, the exact mechanism by which HLA-B27 predisposes to the development of AS still remains an enigma, and may involve hemodynamic features of its protein structure, alterations of its peptidome, potential interactions with gut microbiome, aberrant peptide handling, and associated molecular events [8]. It is also of interest that there are now more than 132 known subtypes of HLA-B27 (HLA-B*27:01 to HLA-B*27:133), encoded by 171 alleles of *HLA-B27* (IMGT/HLA database), but at least one of them (HLA-B*27:06) is definitely not disease associated [8].

The natural function of HLA class I molecules (e.g., HLA-B27) is to bind peptides in the endoplasmic reticulum (ER) and present them on the cell surface to $CD8^+$ cytotoxic

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T lymphocytes. Therefore, it has been proposed that HLA-B27 may be directly involved in the pathogenesis of AS by presenting putative arthritogenic peptides to pathogenic T cells (arthritogenic peptide hypothesis) [9]. This hypothesis suggests that HLA-B27 molecule's presentation of self-derived or nonself-derived peptide(s) can potentially lead to autoreactive $CD8^+$ T cells because of structural resemblance of the peptide(s). These T cells then cause cytotoxicity when exposed to such nonself-peptide(s), possibly derived from gut microbiome, resulting in chronic inflammation [2,10]. Detection of HLA-B27-restricted $CD8^+$ T-cell clones in the synovial fluid and peripheral blood of patients with AS does support this concept [10]. However, the proof of involvement of these 'arthritogenic' peptide(s) directly in AS has not been found as yet. This has led to additional hypotheses that have been proposed to clarify a role for $CD8^+$ T cells in the pathogenesis of AS [11]. A recently reported association of *RUNX3* with AS and psoriatic arthritis further suggests that it is too early to exclude a role for $CD8^+$ T cells because *RUNX3* gene influences the development and maturation of such T cells [12].

> "HLA-B27 misfolding and the unfolded protein response can trigger events that may lead to the pathogenesis of ankylosing spondylitis, such as augmentation of IL-23 production and resultant activation of Th17 cells."

Alternatively, it has been proposed that HLA-B27 may lead to AS via antigen-independent pathways. For example, the folding rate of the nascent HLA-B27 molecules in the ER is relatively slower when compared with other HLA class I molecules. This results in its increased tendency to misfold, and these misfolded molecules tend to be retained in the ER, resulting in activation of unfolded protein response [2,13]. HLA-B27 misfolding and the unfolded protein response can trigger events that may lead to the pathogenesis of AS, such as augmentation of IL-23 production and resultant activation of Th17 cells [2,13].

There are also aberrant forms of HLA-B27 in the ER, such as β2-microglobulin-free B27 heavy chains, as well as novel B27 heavy chain homodimers [13,14]. These aberrant forms can also be expressed on cell surface, where they can evoke an inflammatory response by binding to natural killer (NK) family receptors, including KIRs (killer Ig-like receptors) and/or LILs (leukocyte Ig-like receptors) [14].

ERAP1 & its interaction with HLA-B27

Complex proteins undergo degradation in the cytoplasm to generate peptide fragments that are transported into the ER, where nascent MHC class I molecules typically bind short peptide fragments of approximately 9 residues and transport them to the cell surface for presentation to $CD8^+$ T cells. *ERAP1* is a non-MHC gene located on chromosome 5, and it plays a critical role in processing of peptides prior to their binding to MHC class I molecules for presentation to $CD8^+$ T cells [1,6]. Disruption of the *ERAP1* gene significantly disrupts the presentation of both self- and foreign-derived peptides by HLA class I molecules such as HLA-B27, and thereby interfere with the generation of effective $CD8^+$ T-cell responses [6,15].

As mentioned earlier, *ERAP1* was the second gene discovered (after *HLA-B27*) that increases the risk for AS [1,16]. But most interestingly, this association is restricted to only among HLA-B27-positive patients, suggesting that the pathogenetic role of ERAP1 in AS depends on its functional interaction with HLA-B27 [1,16]. This gene–gene interaction or epistasis between *ERAP1* and *HLA-B27* in AS implicates aberrant peptide handling in the ER that may contribute to the increased risk for AS [1,6,16–20]. ERAP1 can alter the antigen-presenting properties of HLA-B27 molecules, but it may also play a role through some of its other biological properties [6]. Loss-of-function polymorphisms in *ERAP1* could affect peptide supply in the ER and influence misfolding or heavy chain dimerization of HLA-B27. This could lead to an altered surface expression of MHC class I molecules, such as HLA-B27, and this has been suggested as a contributing factor to the pathogenesis of AS [6].

The most recent GWAS results show that, *ERAP2*, which is a member of the ERAP family and has substantial sequence homology with *ERAP1,* is also associated with AS, but in both HLA-B27-positive and HLA-B27-negative patients [1]. *LNPEP*, another member of ERAP family, and *NPEPPS*, which encodes puromycin-sensitive aminopeptidase (a protein that is localized to the cytoplasm and is thought to be involved in processing proteasome-derived peptides before their transport to the ER) are also independently associated with AS [1].

> "Genetic variants associated with reduced function of ERAP1 and loss of expression of ERAP2 are protective for ankylosing spondylitis."

The significant contribution of both *HLA-B27* and the genes belonging to the *ERAP* family as risk factors for AS suggests that changes in the MHC class I antigen processing pathway play an important role in this disease. A higher expression of ERAP1 in dendritic cells of patients with AS when compared with healthy controls suggests that overexpression of ERAP1 is a

mechanism that promotes the pathogenesis of AS [20]. Both protective and susceptible *ERAP1* variants associated with AS have been identified, and the protective variants lead to less ERAP1 activity, and less efficient trimming of HLA-B27 ligands [3,6]. Genetic variants associated with reduced function of ERAP1 and loss of expression of ERAP2 are protective for AS [1].

The association of the four genes belonging to the aminopeptidase family (*ERAP1, ERAP2, LNPEP* and *NPEPPS*) involved in peptide trimming before HLA class I presentation is of particular interest as they may alter the composition and length of HLA-B27 ligands [1,6]. Thus, it has been suggested that these four genes operate in AS "through a quantitative effect on HLA class I peptide presentation or a qualitative effect on the peptide repertoire presented" [1].

Conclusion

Although GWAS have yielded remarkable results in associating different genetic polymorphisms to AS susceptibility [1–3,6], a lot more still needs to be known before all the pieces of this puzzle can be put together to present a clear picture. The epistasis between *HLA-B27* and *ERAP1*, the two genes that together provide the most powerful disease risk factors for AS, implicates aberrant peptide handling in the ER as a contributory factor [19,20]. It is interesting to note that ERAP1 preferentially cleaves hydrophobic amino acids, whereas ERAP2 preferentially cleaves basic residues, and thus ERAP1–ERAP2 heterodimers may also act in concert [1]. It has been hypothesized that ERAP1-mediated HLA-B27 misfolding increases ER stress, driving an IL-23-dependent proinflammatory immune response [2]. But when this hypothesis was recently tested in 49 patients with AS and 22 healthy controls, it was observed that aberrant ERAP1 activity and HLA-B27 carriage does not alter ER-stress levels in patients with AS [20]. This suggests that ERAP1 and HLA-B27 may influence disease susceptibility through some other mechanisms.

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