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SPECIAL ARTICLE

Etiopathogenic role of HLA-B27 alleles in ankylosing spondylitis

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Abstract

HLA-B27 is the major genetic susceptibility factor for ankylosing spondylitis (AS). However, its precise role in the pathogenesis of AS still remains unclear, even though its gene has been cloned and sequenced, and its crystallographic structure has been defined. Arthritogenic peptide and molecular mimicry hypotheses propose mechanisms related to an antigen-presenting function of HLA-B27 to be responsible for disease development. However, peculiar aspects of its immunobiology, such as its misfolding and heavy chain dimerization raise the possibility of involvement of pathogenic mechanisms unrelated to its physiological function. Moreover, HLA-B27 is not a single allele, but a family of 31 different alleles, named HLA-B*2701 to HLA-B*2727. Studies worldwide indicate that the relatively common alleles (subtypes) HLA-B*2705, B*2704, and B*2702 are strongly associated with AS, whereas HLA-B*2706 which is prevalent in South-east Asia and HLA-B*2709 which is prevalent on the Italian island of Sardinia, seem to lack such an association. The distinction between the disease-associated subtypes and those that are not associated, may provide clues to the actual role of HLA-B27 in disease pathogenesis. B*2706 differs from B*2704 by only two residues, and B*2709 differs from B*2705 by only one residue. Moreover, both B*2706 and B*2709 bind an endogenous peptide (derived from vaso-active intestinal peptide type 1 receptor) and also an exogenous peptide (latent membrane protein 2 of Epstein-Barr virus) but in two drastically diverse conformations. These recent X-ray diffraction studies of individual peptides in the context of different HLA-B27 alleles broaden our perception of the possible pathogenetic role of this molecule in the development of AS and related spondyloarthopathies. In summary, the pathogenetic role of HLA-B27 in AS seem to be quite heterogenous, and cannot be explained by a single mechanism, and new ideas have been raised based on the aberrant immunobiologic features of HLA-B27.

Key words: alleles, ankylosing spondylitis, etiopathogenesis, genetics, HLA-B27, polymorphisms, spondyloarthropathies, subtypes.

The remarkable association of HLA-B27 with ankylosing spondylitis (AS) has been known for over 30 years.[1,2] Although studies on monozygotic and dizygotic twins suggest that the disease risk attributable to HLA-B27 is only approximately 16%,[3] it is abundantly clear that the B27 gene is the major genetic susceptibility factor for this disease. Yet, despite many years of intense research, its role in disease pathogenesis remains unexplained. However, numerous hypotheses have been put forward.[4–7]

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HLA-B27 STRUCTURE

The three-dimensional crystalline structure of HLA-B27 has been delineated, and its gene has been cloned and sequenced.[8,9] The crystallographic study of these molecules revealed a structure designed to carry peptide

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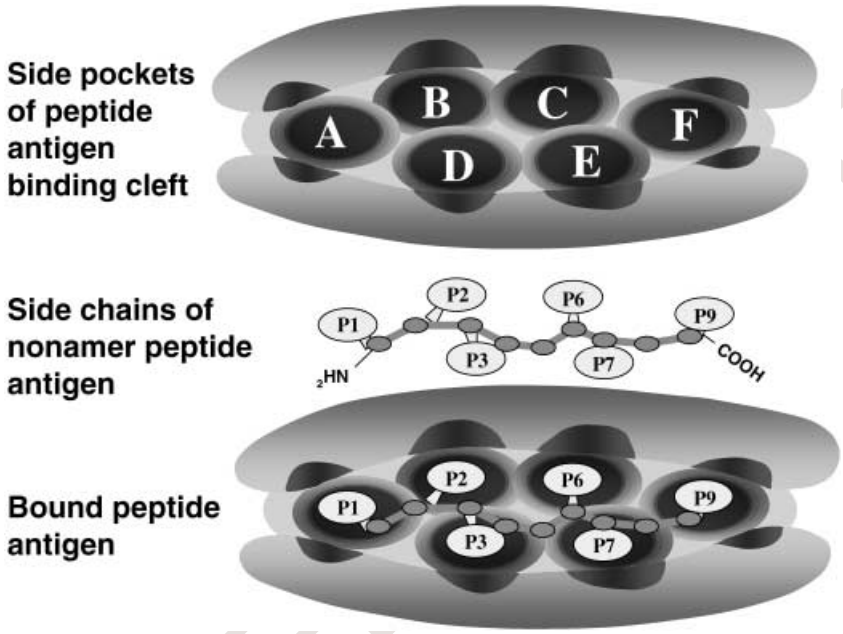

Figure 1 Peptide-binding pockets of an HLA class I molecule. Crystallographic studies of HLA class I molecules have indicated a series of pockets (A to F) within the binding cleft that accommodate the amino acid side-chains of the bound peptide.[10–15] The amino acid residues are named P1, P2, P3, etc. starting at the amino terminus of the peptide. The principle anchoring residues for peptides bound by HLA-B27 are P2 and P9, which are accommodated by binding pockets B and F, respectively. Figure reproduced from reference[6] (permission requested).

antigens in a binding cleft formed by the alpha 1 and alpha 2 domains of the heavy chain.[10–15] This binding cleft has six side-pockets designated by the letters A through F that accommodate the side chains of the amino-acids of the bound peptide[13,14] (Fig. 1).

Polymorphic residues of the HLA molecule involved in the formation of these pockets influence the specific peptides that are bound. Peptides eluted from HLA class I molecules have been shown to be restricted in length (primarily 9 or 10 amino acid residues) and to have a sequence motifs characteristic of the particular HLA allele or family of alleles.[15]

The individual amino acid residues of the bound peptides starting from the amino terminal end of the peptide are referred to as P1, P2, etc., so that the C-terminal residue is P9 in the usual nine-residue peptide. The residues at P2 and P9 form the two principle anchors for binding of the antigenic peptides that bind in the antigen-binding cleft of HLA-B27.

THE PATHOGENIC ROLE OF HLA-B27

The 'arthritogenic peptide' hypothesis proposes that AS results from the ability of HLA-B27 to bind a unique antigenic peptide(s) with resultant HLA-B27 restricted cytotoxic (CD8+) T-cell response targeted against antigen(s) found at sites involved in this disease.[16] A modification of the original arthritogenic peptide hypothesis proposes that disease results from a breakdown of self-tolerance by initial HLA-B27-restricted presentation of peptide(s) derived from putative triggering pathogen(s).

Another hypothesis proposes molecular mimicry where cross-reactive cellular/humoral immune response to microbial epitopes cross-reacts with HLA-B27. This results in either T-cell repertoire deletion or makes the B27 peptide complex (B27 heavy chain/beta 2 microglobulin/bound peptide) become a target of autoreactive T cells. HLA-B27-derived peptides may themselves elicit immune response if there is loss of immune tolerance.

Yet another hypothesis proposes that HLA-B27 may modify microbial handling and impair immunity, resulting in defective immune response and inefficient elimination of the triggering pathogen(s). There is, however, increasing evidence that the pathogenetic role of HLA-B27 may be unrelated to its physiologic function. Thus this modulation of the inflammatory response may be mediated, not by antigen-presenting function, but by nonantigen presenting function of HLA-B27.[17]

Like any other MHC-class I molecule, HLA-B27 functions well in presenting peptides to CD8+ T-lymphocytes, and thus, for example, it provides strong protective immune response to influenza, and some of the other viruses, including HIV. Antigen recognition by CD8+ cytotoxic T-cells is dependent upon a number of critical steps in MHC class I antigen processing. These include proteosomal cleavage of the antigen, TAP transport of

the antigenic peptides into the endoplasmic reticulum and MHC class I binding, and finally the expression of the MHC-bound peptide on the cell surface.[18] Studies by Colbert *et al*.[19] suggest that the pathogenetic role may be a consequence of the unusual tendency of HLA-B27 molecule to misfold and thus get retained in the endoplasmic reticulum leading to degradation in the cytosol. This misfolding and its consequences, rather than allele-specific arthritogenic peptide presentation, may be the pathogenetic mechanism of B27-associated diseases.

Heavy chain homodimer formation may occur in the endoplasmic reticulum as a result of the slower-folding kinetics of HLA-B27, and it involves not only cysteine at position 67 but also the conserved structural cysteine at position 164.[20,21] Such homodimers have been detected within the endoplasmic reticulum and at the cell surface. Thus HLA-B27 can be expressed on the cell surface in some non-conventional forms, and the presence of such aberrant forms of HLA-B27 has lead to new avenues of research to explain its pathogenetic role. These B27 heavy chain homodimers can act as ligands for a number of NK and related immunoreceptors, expressed on NK cells, T and B-lymphocytes, and members of the monocyte/macrophage lineage.[22,23] Such homodimers are expressed in B27-transgenic rodent models, and are ligands for paired Ig-like receptors.[24]

MHC restriction indicates that CD8+ T-cells conventionally interact with MHC class I molecules and CD4+ T-cells interact with MHC class II molecules. Boyle *et al*.[25–27] have looked for any errant behaviour of T-cells isolated from patients with AS to understand the pathogenesis of this disorder. They were able to isolate from three different patients with AS, all B27 positive, CD4+ T-cells that interact with B27, an MHC class I molecule. This finding breaks the conventional rules of MHC restriction. These CD4+ T-cells appear to recognize an unconventional form of B27 in that some recognized unfolded heavy chain homodimers, while others recognize empty heterodimers. Such cells could not be detected in the blood of B27-positive healthy individuals. It is speculated that the CD4+ T-cells may be reacting to non-conventional forms, specifically B27 heavy chain homodimers, which might mimic MHC class II molecules and are recognized by CD4+ T-cells. Therefore, it is possible that continual interaction between the two could trigger or modulate T-cell effector functions that may initiate the disease process. It is of interest that HLA-B27 transgenic animal models implicate CD4+ T-cells in disease pathogenesis. Thus, in the past few years the focus has shifted from the one centred largely on the physiological peptide-presenting function of HLA-B27, to include ideas based on aberrant aspects of its immunobiology.[28]

HETEROGENEITY OF HLA-B27: 31 DIFFERENT ALLELES

HLA-B27 is not a single allele, but a family of at least 31 different alleles, named HLA-B*2701 to HLA-B*2727 (Table 1).[29] They seem to have evolved from the most widespread subtype, B*2705.[30,31] HLA-B*2705 comprises six alleles (B*27052, B*27053, B*27054, B*27055, B*27056 and B*27057) that differ only by silent mutations. B*27052 differs from B*2713 only in the leader segment of the gene, which is not expressed, and therefore the molecule encoded by these two alleles is identical. One of the allele assignments – HLA-B*2722 – has been withdrawn when subsequent studies showed its identity as HLA-B*2706. The distribution of the subtypes of HLA-B27 shows a wide variation across populations[32–53] (Table 1).

These subtypes differ among each other by one or a few amino acid changes, and they bind overlapping peptide repertoires. It is a matter of question whether

Table 1 Listing of the currently recognized 31 alleles (subtypes) of HLA-B27[29]

B*2701†		B*2712
B*2702		B*2713
B*2703‡		B*2714†
B*2704		B*2715
B*2705	B*270502	B*2716
	B*270503	B*2717
	B*270504	B*2718
	B*270505	B*2719†
	B*270506	B*2720
	B*270507	B*2721
B*2706¶		B*2723
B*2707§		B*2724
B*2708		B*2725
B*2709¶		B*2726
B*2710†		B*2727
B*2711		

Note that HLA-B*2722 is not listed as this designation has been withdrawn when subsequent studies showed its identity to HLA-B*2706. The B27 alleles shown in bold type are associated with AS. †The disease occurrence in at least one patient with these alleles was shown, but needs association studies. ‡Disease association is controversial.[53,77] §Suggested to be disease associated; but not in Greek Cypriots. ¶These alleles seem not to have an association with the disease.

or not all HLA-B27 subtypes carry risk for the disease and whether HLA-B27 subtypes provide similar or different levels of disease risk (Table 1). Worldwide studies indicate that HLA-B*2702, B*2704, and B*2705 are strongly associated with AS.[30] B*2707 has also been suggested as be associated with the disease[30] but not in Greek Cypriots.[40] However, two subtypes, HLA-B*2706 and HLA-B*2709, seem to lack an association (Table 1).[54] There is only very limited information about the most recent subtypes. Presence of at least one case of AS or related SpA has been documented in individuals possessing any of the first 10 subtypes, B*2701 to B*2710, B*2714 and B*2719 (Table 1),[30] but an association with any of these subtypes needs to be confirmed by proper epidemiologic studies. Lack of apparent disease association with some of the other more recently described rare subtypes, may be mostly due to the rarity of these alleles, rather than to their possible lack of disease association.

DISEASE ASSOCIATED HLA-B27 SUBTYPES IN ASIAN POPULATIONS

HLAB* 2705 is the most widespread B27 subtype and is clearly associated with AS and related SpA around the world, except perhaps among the West African populations of Senegal and Gambia.[30,53,55,56] The sequence variation of the B27 subtypes and their pattern of worldwide distribution suggest that B*2705 might be the ancestral subtype from which others have evolved.[30,57,58] It is almost the only subtype observed among the native populations of eastern Siberia and North America, and is present in approximately 90% of the B27-positive individuals of northern European extraction.[59,60] Its frequency is 5–10% in South-east Asia.[32,43,61] One presumes that B*2705 subtype is mostly the B*270502 allele; the other alleles (B*270503, B*270504, B*270505, B*270506 and B*270507) have only more recently been described. As noted previously, the B*270502, B*270503, B*270504 and B*2713 alleles produce the same mature B27 molecule at the cell surface.

HLA-B*2704 which is also strongly associated with AS and related SpA is the predominant subtype in Asia among the Chinese and Japanese, and is quite prevalent in south-eastern Asian populations except native Indonesians and Malays.[32,38,43,51,57] It may be the progenitor from which B*2706 and other, rare, Asian subtypes have arisen (such as B*2711, B*2715 and B*2720). No clinical studies of disease association with these rare alleles have been reported.

HLA-B*2706 LACKS ASSOCIATION WITH ANKYLOSING SPONDYLITIS IN SOUTH-EAST ASIAN POPULATIONS

In Thailand, where B*2704 is slightly less prevalent than B*2706 (Table 2), 91% of B27-positive AS patients possess the B*2704 allele versus 47% of B27-positive normal controls.[56,62] Most interestingly, B*2706 was totally absent among patients but was present in 47% of B27-positive normal controls.[56,62] Subsequent studies from Indonesia,[50,63] Singapore[46] and Taiwan[64] have confirmed the lack of association of B*2706 with AS. Nevertheless, a report of two B*2706 patients from mainland China[56] where this subtype is very infrequent, might be consistent with some effect of this allele in disease susceptibility, at least, in this population. However, this observation needs to be independently validated and needs an independent study of large populations of Chinese B27-positive patients and healthy controls. It is interesting that B*2706 differs from B*2704, the disease associated subtype, only at residues 114 and 116.[46,50,62,63,65,66]

HLA-B*2709 LACKS ASSOCIATION WITH ANKYLOSING SPONDYLITIS IN SARDINIA

HLA-B*2709 is primarily observed among Italians residing on the island of Sardinia, and it differs from the disease-associated subtype B*2705 by a single amino acid substitution: aspartic acid at position 116 is substituted by histidine.[67] It appears to show a very weak association with SpA compared to the other two subtypes, B*2705 and B*2702, that are present in the population. In a study of the island population of Sardinia, B*2709 was present in 10/40 (25%) healthy B27-positive controls but in none of the 50 B27-positive AS patients.[67] However, four B*2709-positive undifferentiated SpA patients have been reported from the Italian mainland, where the general prevalence of B*2709 is very much less common than in the Sardinian population. One of these patients had oligoarthritis and sacroiliitis.[68] The next reported patient had peripheral arthritis, peripheral enthesitis, and dactylitis without sacroiliitis or axial involvement.[69] Two additional patients with undifferentiated SpA who were B*2709-positive were identified in a case control study by the same group.[37]

HLA-B27 subtyping has recently been extended to 70 AS patients from Sardinia and 70 from the Italian mainland, and none of them was found to be B*2709-positive.[68]

Table 2 HLA-B27 subtype frequencies in different world populations

Populations	*2701	*2702	*2703	*2704	*2705	*2706	*2707	*2708	*2709	*2711	*2713	*2714
Northern Europe[32]		10			90							
Denmark,[33]		10			90							
Southern Europe[32]		20			80							
Spain (Galicia)[34]		18			80			3				
Northern Spain[35]		7			91		1				1	
Azores[36]			7		86		7					
Italy[37]		38			50		4		7			
Sardinia[38]					75				25			
Greece[39]		34	8		50		8					
Cyprus (Greeks)[40]		52			32		17					
Turkey[41]	7	30			43		14	5				
Lebanon[42]		24	12		35		30					
Jewish[43]		48		3	38		13					
Siberia[43]		14			84							2
Northern India[43]				33	61		6					
Western India[44]				34	34		18	12				2
Japan[45]				82	18							
Chinese[43]		2		66	31		2					
Singapore (Chinese)[46]				89	2	9						
Taiwan[47]	0.05	0.5	3	87	4	7	2	0.02		0.02		
Taiwan (Han-Chinese)[48]				94	6							
Taiwan (Aborigines)[48]				100								
Chinese Indonesian[49]				38		62						
Native Indonesians[49]				6	6	89						
Malays[51]				19	6	72	3					
Thailand[43]				42	5	53						
Maoris[43]				36	64							
Brazil[52]		10	6		80		3				< 1	
North Africa[32]		50			50							
West Africans[53]			32		68							

Numbers are rounded off for simplicity and indicate percentages in healthy controls.

These data suggest that B*2709, like B*2706, is not associated with AS, although it may confer some susceptibility to other forms of SpA.

The distinction between the disease-associated subtypes and those that are not associated may provide clues to the actual role of HLA-B27 in disease pathogenesis. As previously mentioned, B*2709 differs from B*2705 only in residue 116 of the heavy chain (His in B*2709 and Asp in B*2705), and HLA-B*2706 differs from B*2704 by amino acid changes at only two residues, including the residue 116. These differences lead to differential binding of peptides to these subtypes. B*2709 shares 79% of its peptide repertoire with B*2705[70] and B*2706 shares 88% of its repertoire with B*2704.[71] The differential subtype association with disease seems to be related to differentially bound peptides and not due to altered antigenicity of shared ligands.[70,71]

Recent studies have provided alternative, and potentially more exciting explanations for the differential disease association between B*2705 and B*2709. Starikov *et al.*[72] have performed molecular dynamics simulations and shown increased flexibility of a model peptide 'm9' (GRFAAAIAK) in the groove of the B*2709 subtype than in that of B*2705, despite its essentially identical conformation in either subtype which had been previously shown crystallographically.[73] Hulsmeyer *et al.* have examined the crystal structures of B*2705 and B*2709 molecules with an endogeneous peptide, pVIPR (RRKWRRWHL, derived from vasoactive intestinal peptide type 1 receptor [residues 400–408]) which fits some criteria for an arthritogenic peptide.[28] They found that this peptide can bind to the two subtypes differently. It binds to B2709 in a conventional conformation; but to B2705 in two different conformations:

conventional and non-conventional. The alternative conformation of the pVIPR peptide binding to B*2705 appears to affect T-cell receptor recognition and this may lead to the emergence of distinct T-cell repertoires in individuals with these subtypes. This may explain the higher frequency of pVIPR-reactive CD8+ T-cells previously described in subjects with the B*2705 compared to those with the B*2709.[74]

Similar to the pVIPR, the viral peptide pLMP2 (RRRWRRLTV, derived from latent membrane protein 2 [residues 236–244] of Epstein-Barr virus) is presented by the B*2705 and B*2709 molecules in two drastically deviating conformations.[75] This indicates that a pathogen-derived peptide can exhibit MHC class I subtype-dependent, drastically distinct binding modes. The viral peptide pLMP2 also shows high sequence homology to the self-peptide pVIPR. However, extensive structural similarity is observed only when the peptides are presented by B*2705 and not when presented by B*2709. These results raise the possibility that molecular mimicry between pLMP2 and pVIPR in the HLA-B27 context may be a possible mechanism that may contribute to AS. Moreover, it is an allele-dependent property and may account for the differential association of the HLA-B*2705 and B*2709 with AS.[75] Lopez de Castro[76] has recently reviewed the implications of the recent X-ray diffraction studies of HLA-B27 containing antigenic-bound peptides involving viral epitopes that are immunodominant in HLA-B27-restricted T-cell responses against Epstein-Barr, influenza and HIV viruses. He concluded that antigen presentation by B27 molecule can be largely influenced by polymorphism in the F pocket where residue 116 is located and that microbial peptides presented by HLA-B27 may have a pro-inflammatory effect through increased NK activation.

In summary, the pathogenetic role of HLA-B27 in AS seems to be quite heterogenous, and cannot be explained by a single mechanism. New ideas are raised based on the aberrant immunobiologic features of HLA-B27. These include increased misfolding of the heavy chains inside the endoplasmic reticulum, with resultant stress response and expression of aberrant forms of HLA-B27 on the cell surface. These aberrant forms, specifically B27 heavy chain homodimers, may mimic MHC class II molecules and can thus be recognized by CD4+ T-cells. Different subtypes of HLA-B27, show a differential association with AS, although they differ among each other by only one or a few amino acid changes. The distinction between the disease-associated subtypes and those that are not associated may also provide clues to the actual role of HLA-B27 in disease pathogenesis. Recent X-ray diffraction studies of individual peptides in the context of different HLA-B27 alleles shed more light on the pathogenesis of AS and also raise the possibility that molecular mimicry between pLMP2 and pVIPR in an allele-dependent manner may be a possible mechanism that may contribute to AS.

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