

HLA and the Spondyloarthritis

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ABSTRACT The Spondyloarthritis are a group of diseases with a strong tendency for family aggregation, which includes mainly Ankylosing Spondylitis (AS), Reiter's Syndrome / Reactive Arthritis (ReA), Enteropathic Spondylitis (Crohn's disease and Ulcerative Colitis), Psoriatic Arthropathy (PsA), and Undifferentiated Spondylitis (uSpA). The axial skeleton, mainly the sacroiliac joints, the peripheral joints – more frequently in the lower limbs, and the tendon insertions (entheses), are particularly prone to an inflammatory process that may involve several targets at the same time or sequentially. Main symptoms depend on the stage of the disease or the SpA subset under examination, and their overlap is frequent. The most known subset of the SpA is Ankylosing Spondylitis, a chronic systemic inflammatory disease of the axial skeleton whose etiology is still unknown, affecting always the sacroiliac, and usually the apophyseal, costovertebral, and costotransverse joints of the spine. The symptoms begin in late adolescence or early adulthood, and chronic inflammatory back pain and stiffness are the most common and characteristic initial presenting complaints in adult-onset AS. The description in 1973 of a very strong association between HLA-B27, an immune response gene and AS permitted to consider this disease to have an autoimmune pathogenesis. The association of AS with HLA-B27 may be 95% to 99% according with the majority of authors but this proportion is inferior in Reiter's Syndrome and Psoriatic Arthritis.

INTRODUCTION

The Spondyloarthropathies (SpA)

The Spondyloarthritis (SpA) are a group of diseases with a strong tendency for family aggregation, which includes mainly Ankylosing Spondylitis (AS), Reiter's Syndrome / Reactive Arthritis (ReA), Enteropathic Spondylitis (Crohn's disease and Ulcerative Colitis), Psoriatic Arthropathy (PsA), and Undifferentiated Spondylitis (uSpA). These and other conditions (summarized in Table 1) share a number of features (Calin 1998). The axial skeleton, mainly the sacroiliac joints, the peripheral joints – more frequently in the lower limbs, and the tendon insertions (entheses), are particularly prone to an inflammatory process that may involve several targets at the same time or sequentially. Main symptoms depends on the stage of the disease or the SpA subset under examination, and their overlap is frequent. The most known subset of the SpA is Ankylosing Spondylitis, a chronic systemic inflammatory disease of the axial skeleton whose etiology is still unknown, affecting always the sacroiliac, and usually the apophyseal, costovertebral, and

costotransverse joints of the spine. The symptoms begin in late adolescence or early adulthood, and chronic inflammatory back pain and stiffness are the most common and characteristic initial presenting complaints in adult-onset AS (Khan 1998). The description in 1973 of a very strong association between HLA-B27, an immune response gene and AS, suggested an autoimmune pathogenesis

Table 1: Individual conditions that overlap to form the spondylarthritides

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- . Ankylosing Spondylitis.
 - . Reiter's syndrome / reactive arthropathy (Campylobacter, Yersinia, Shigella, Chlamydia spp).
 - . Enteropathic spondylitis (Crohn's disease and ulcerative colitis).
 - . Psoriatic arthropathy.
 - . Uveitis.
 - . Juvenile ankylosing spondylitis.
 - . Seronegative enthesopathic arthropathy syndrome.
 - . Undifferentiated spondylitis (i.e. subset of patients who have spondylarthropathic features but fail to meet criteria for ankylosing spondylitis, Reiter's syndrome, or other conditions, e.g. dactylitis, uveitis plus unilateral sacroiliitis).
 - . Pustulotic arthro-osteitis (considered by the Japanese to be part of spondylarthropathy spectrum but rare in USA and Europe).
 - . Behçet's disease
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(Schlosstein et al. 1973; Brewerton et al. 1974). The association of AS with HLA-B27 may be 95% to 99% according to the majority of authors. This proportion is lower in Reiter's Syndrome and Psoriatic Arthritis (Calin 1998).

Structure and Function of HLA Molecules

The two main groups of HLA molecules – Class I (HLA-A, HLA-B and HLA-Cw) and Class II (DR, DQ and DP) – are encoded in the short arm of chromosome 6, a segment of DNA with four megabases in length that contains approximately 200 genes. Class I molecules have α -chain with five domains ($\alpha 1$ and $\alpha 2$ – the peptide binding domain; $\alpha 3$ – the immunoglobulin-like domain; the transmembrane domain; the cytoplasmic tail), and a β chain which is encoded in the chromosome 15 – the $\beta 2$ – microglobulin gene. They can be identified in the majority of somatic cells but with different levels of expression depending on the specific tissue under observation. Class II molecules are fully encoded by genes in chromosome 6 and have four domains: the peptide binding domain ($\alpha 1$ or $\beta 1$), the immunoglobulin-like domain ($\alpha 2$ or $\beta 2$), the transmembrane region, and the cytoplasmic tail. These molecules are expressed by a subgroup of immune cells that includes B-cells, activated T-cells, macrophages, dendritic cells and thymic epithelial cells. The function of HLA Class I and II molecules is to present short pathogen-derived peptides to T-cells, initiating the adaptive immune response (Klein and Sato 2000). The peptide binding groove of the HLA molecule between $\alpha 1$ and $\alpha 2$ domain in class I, and $\alpha 1$ and $\beta 1$ in class II, is formed by six pockets (A-F) surrounding the binding site, each one with the capacity of interacting with individual amino acids in the peptide. Both A and F pockets are particularly important since they are, respectively, the binding sites for the amino and carboxyl ends of the peptide. These pockets are highly conserved among HLA molecules. Pocket differences provide allele-specific anchor sites for the binding of particular amino acids. The side chains of amino acids residues in the middle of the peptide protrude out of the groove, whereas those of most of the remaining residues point into the groove and are housed in the pockets. Polymorphic residues of the HLA molecule involved in the formation of pockets influence the specific peptides that are bound.

The length of the peptides bound to HLA class I is restricted (9 or 10 amino acids) and they have sequence motifs that are characteristic of a particular HLA allele or family of alleles. Individual residues of the peptides are named as P1, P2, etc; beginning from the amino terminal end (P1) and finishing in the C-terminal end (P9) in the usual 9 amino acids long peptide (Jardetzky et al. 1991). Pockets B and F are particularly important because the side chains that fit into them serve as peptide's anchors, and this feature determines which peptide is bound to the molecule. The remaining pockets are less important in the choice of binding specificity. In the case of HLA-B27, the majority of binding peptides have the residue arginine at P2, since the side chain of this amino acid fits well in the B pocket of the molecule. B pocket's residues include His9, Thr24, Glu45 and Cys67. The arginine of P2 shows affinity to B pocket of B27 because of the critical residues Glu and Cys at positions 45 and 67. Among different subtypes of HLA-B27, B*2718 and B*2723 are distinct because they have no Cys67. This is particularly important, since this lack modifies the B pocket and consequently the peptide binding preferences. In pocket F the critical residues involved are at positions: 77, 80, 81, 97 and 116. The peptide binding preference for this pocket is not so restricted as in the case of B pocket, because the positions are more polymorphic. The other binding pockets act as secondary peptide anchor positions and their contribution results of the interaction with B and F pockets (Alvarez and Lopez de Castro 1999; Lamas et al. 1999; Martinez-Borra et al. 2000).

HLA-B27 Subtypes and Disease Association

Besides its prominent role in the development of the disease, HLA-B27 molecule is also present worldwide in healthy people. HLA-B27 is present throughout Eurasia but is virtually absent among the genetically unmixed native population of South America (Amerind tribes), the Bantus and Sans (Bushmen) of Equatorial and Southern Africa, and the aborigines of Australia. The prevalence of HLA-B27 is very high in native populations of North America, with an extreme of 50% found among the Haida Indians, living on the Queen Charlotte Islands of the Canadian province of British Columbia (Gofton 1980; Khan 1998). The prevalence of B27 ranges between 2 and 18% among Caucasoids. There are 27 HLA-

B27 different alleles however HLA-B*27052, B*27053 and B*27054 subtypes differ uniquely by silent mutations; and B*2722 was removed from HLA database, since its sequence was identical to B*2706. HLA-B*2705 is the most widespread subtype around the world, and the structural patterns and ethnic distribution

suggest that it might be the ancestral subtype from which others have evolved. This evolution consisted in one or few genetic events, usually gene conversion and point mutations (B*2703). HLA-B27 subtypes were classified according to their structural features. Three groups can be distinguished (Table 2) (Fig. 1). All the subtypes

Table 2: Classification of HLA-B27 subtypes (Ramos M, Lopez de Castro JÁ, 2002)✧.

Group	Subgroup	Subtype
1	1 ^A	B2713
	1B	B2703, B2727
	1C	B2709, B2710
2	2 ^A	B2701, B2702, B2708✦, B2712✦, B2716✦, B2723
	2B	B2707, B2714, B2719
3	3 ^A	B2704, B2718,
	3B	B2711
	3C	B2706, B2715, B2720, B2721□, B2724, B2725

✧ This classification is based on the evolutionary relationship with B2705

✦ The precise evolutionary relationship of these three subtypes is unclear

□ B2721 might also have evolved from B2706

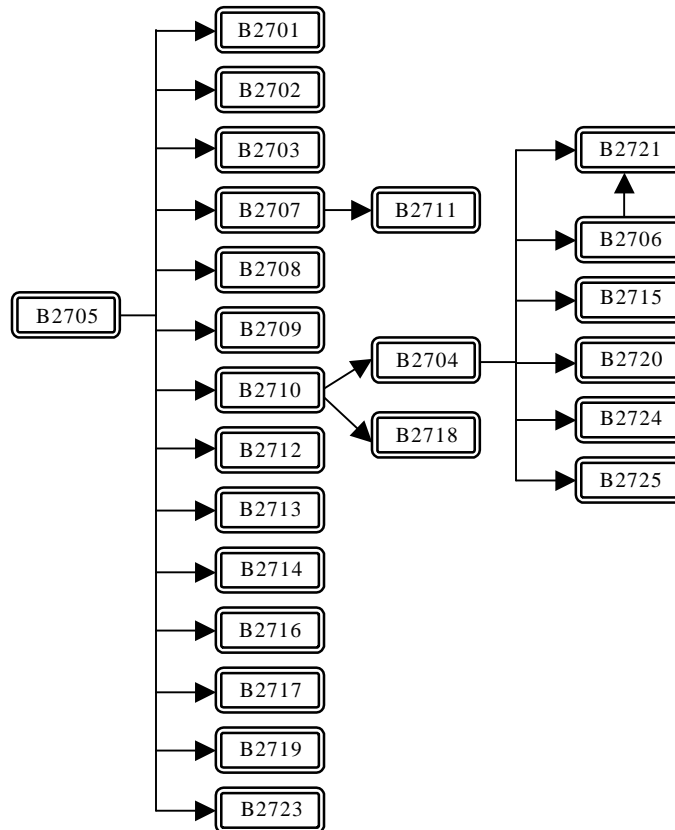


Fig. 1. Evolution of HLA-B27 subtypes. Different colours are used to distinguish the predominant subtypes of each ethnic group. Caucasoids: blue; Orientals and Pacific Islanders: red; Blacks: grey; North Amerindians: pink; unknown: green. (Ramos M, Lopez de Castro JÁ, 2002)

included in group 1 diverged from B*2705 by a single amino acid change, caused by a single genetic event (point mutation or gene conversion) in the leader sequence (1A), α 1 chain (1B) or α 2 chain (1C). Group 2 subtypes are different from B*2705 in multiple amino acids in the same domain, probably inherited by single gene conversion. Subtypes with changes in α 1 are included in subgroup 2A, and in α 2 are included in 2B. Group 3 is constituted by subtypes that are related with other alleles than B*2705. These subtypes are derived by single genetic events that have introduced one or more amino acid changes in a single domain. It is formed by three subgroups: 3A (subtypes derived from B*2710), 3B (subtypes derived from B*2707) and 3C (subtypes derived from B*2704) (Ramos and Lopez de Castro 2002).

As Table 3 shows, HLA-B27 subtypes differ from B*2705 in some amino acids substitutions in the sequence leader, α 1, α 2 and α 3 domains. It is well established that these subtypes are distributed in different ethnic groups, which may be the result of genetic and geographic origins. There is evidence through case-control studies, that B*2705, B*2702, B*2704, B*2707 are associated with AS (Gonzalez-Roces et al 1997; Ramos and Lopez de Castro 2002). B*2705 is present in nearly all populations of the world but it is over-represented in the Circumpolar and Subarctic regions of Eurasia and North America. In the Euro-Caucasoid population it was identified in approximately 90 to 96% of B27 healthy individuals and patients with AS and related spondyloarthropathies (Khan 1995). B*2702 is restricted to Caucasoids where it is the predominant subtype among Middle East (Jewish) and North African-Caucasian (Algerians and Tunicians) populations. It accounts for 5-10% of HLA-B27 Euro-Caucasians, Mestizo Central and South American populations. HLA-B*2704 is strongly associated with SpA, accounting for 55% of the B27 alleles, and it is the predominant subtype among Chinese and Japanese (Lopez de Castro 1989). B*2707 has been described in Asian populations (Indians, Chinese and Thais) at low frequency (5.8%) but it has recently been detected in a higher frequency (18%) in Western Indians (Lopez-Larrea et al. 1995; Shankarkumar et al. 2002). HLA-B*2701 is a very rare subtype and until now it was only reported in a single Caucasoid kindred associated with the disease (MacLean 1992;

Gonzalez-Roces et al. 1994). B*2703 has been observed among West Africans (Senegal, Gambia and Mali) and on rare occasions, outside of Africa but only among African descendants. Its association with the disease it's unclear. Hill et al. (1991) refers that unlike the other HLA-B27 subtypes, B*2703 may not be associated with ankylosing spondylitis. A study carried by Brown et al. (1997) points that neither HLA-B*2703 nor HLA-B*2705 are associated with AS in a Gambian group, which probably means that this population has unknown genetic protective factors. However, disease association with B*2703 allele was identified in Senegalese population, suggesting that this allele may be a susceptibility factor for AS (Gonzalez-Roces et al. 1997; Ramos and Lopez de Castro 2002). B*2706 and B*2704 are very similar, differing only in two amino acids. Like B*2704, B*2706 was also identified in Asia. It is over-represented in Thailanders and absent in the Asian Indian populations. Interestingly, this allele is not associated with AS in Thailand (Gonzalez-Roces et al. 1997), Indonesia (Nasution et al. 1997) and amongst Singapore Chinese (Ren et al. 1997). B*2708 is a rare subtype which was initially observed in two healthy individuals from the United Kingdom (Brown et al. 1996). This subtype was associated with disease (AS) for the first time in a large affected family from the Azores (Armas et al. 1999), and subsequently, it was found in patients with Psoriatic Arthritis in Spain (Gonzalez et al 2002), in a group of patients from Ankara (Turkey) (Ozgul et al. 2002) and in an American family of European descent. Recent studies from Western India, reported a high frequency of B*2708 (12%), and the association of this allele with chronic arthritis in severe haemophilia (Ghosh et al. 2003; Shankarkumar et al. 2003). HLA-B*2709 is only found in Sardinia where it accounts for approximately 20% of B27 positive individuals. Although its restricted ethnic distribution, the fact that this allele has not yet been linked to disease, suggests a negative association between B*2709 and AS (D'Amato et al. 1995). HLA-B*2710 and HLA-B*2711 have been identified in an American Caucasian family with spondylitis and in a healthy B27 positive Japanese women, respectively (Khan 1998; Hasegawa et al. 1996). B*2712 was identified in a healthy family in Spain and another in the United Kingdom. Despite the differences in residues 69, 70 and 71, B*2712 is

Table 3: Amino acid changes among HLA-B27 subtypes (Ramos M, Lopez de Castro JÁ, 2002)

Subtype	Residues Number										α2										α3									
	-20	59	63	67	69	70	71	74	77	80	81	82	83	84	94	95	97	103	113	114	116	131	143	152	156	163	211			
2705	A	Y	E	C	A	K	A	D	D	T	L	L	R	T	L	L	N	V	Y	H	D	S	T	V	L	E	A			
2701	nd	-	-	-	-	-	Y	N	N	I	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
2702	-	H	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
2703	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
2704	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	S	-	H	D	Y	Y	R	-	-	-	G			
2706	nd	-	-	-	-	-	-	-	S	N	R	G	-	-	-	-	-	-	-	N	Y	-	-	-	-	-	G			
2707	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
2708	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
2709	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	H	-	-	-	-	-	-			
2710	nd	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	S	-	H	N	Y	R	-	-	-	-	nd			
2711	-	-	-	-	-	T	N	T	S	N	R	G	-	-	-	-	-	-	-	N	Y	-	-	-	-	-	-			
2712	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
2713	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	-	-	-			
2714	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	W	T	-	-	-	-	-	-	-	-	-	nd			
2715	nd	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E	-	-	nd			
2716	nd	-	-	-	T	N	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	nd			
2717	nd	F	-	-	-	N	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	nd			
2718	-	-	-	S	T	N	T	Y	S	N	R	G	-	-	-	-	-	-	-	-	-	-	-	E	-	-	nd			
2719	nd	-	-	-	-	-	-	-	S	-	-	-	-	-	I	I	R	-	-	-	-	-	-	-	-	-	nd			
2720	nd	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	R	-	H	N	Y	R	-	-	-	-	nd			
2721	nd	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	D	Y	-	-	-	-	-	nd			
2723	nd	-	N	F	T	N	T	Y	S	-	-	-	-	-	-	-	S	-	H	N	Y	R	-	-	-	-	nd			
2724	nd	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	N	Y	R	S	E	-	-	nd			
2725	nd	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E	W	L	nd			

(-) indicate identity with B*2705

nd= not determined

very similar to B*2708 (Balas et al. 1998). B*2713 was identified in a healthy control family in a population from Northern Spain, and it has not been reported to be represented in other ethnic groups (Gonzalez et al. 2002). Recently B*2714 was identified in association with AS in Western India (Shankarkumar et al. 2003). The remaining subtypes are extremely rare and there are no control-studies relating them to disease, despite the occurrence of some of them in sporadic cases of AS (Kim et al. 2000; Steiner et al. 2001; Tamouza et al. 2001; Darke et al. 2002; Fieldman et al. 2002; Gans et al. 2002; Voorter et al. 2002; Steiner et al. 2003).

Other HLA Alleles Possibly Associated With SpA

The association of B27 with AS remains the strongest among any immunogenetic disease. However, only a small proportion of B27 positive individuals develop the disease, and B27 negative manifest the classical features of AS. These facts suggest that B27 is not the only gene involved in the pathogenesis of SpA. The extensive linkage disequilibrium that exists among MHC genes complicates the identification of any possible gene involved. Two HLA class I genes, B39 and B60, have been associated with the susceptibility to AS. Structural differences and different peptide-binding characteristics exist between these alleles and B27. HLA-B39 shares with B27 important B pocket amino acids including: glutamic acid, cysteine and tyrosine but not lysine which is characteristic of B27 allotypes. B39 and B27 bind preferentially to positively charged amino acids at P2, including arginine. On the other hand, B60 preferentially binds glutamine at P2 suggesting that B60 either presents different “arthritogenic peptides”, or acts in a different way to B27 in increasing susceptibility to AS (Wordsworth and Brown 1998). Robinson et al. (1989) first reported that the association between HLA-Bw60 and HLA-B27 increases the susceptibility to AS. In another study, the risk of developing AS triplicated in heterozygotes for HLA-B27 and HLA-B60, when compared with heterozygotes for HLA-B27 and another HLA-B allele (Feltkamp 1996). These results were confirmed in the UK (Brown et al. 1996) but were not consistent between all ethnic groups (Reveille et al. 1994; Yamaguchi et al.

1995) and may represent the presence of a further susceptibility marker with a differential strength of linkage disequilibrium with B60 in different populations (Wordsworth and Brown 1998). A strong association between HLA-B39 and AS among HLA-B27 negative patients was reported in patients. In fact, eight patients were B27 negative and three of them were B39, which represents a significantly higher association, when compared with HLA-B27 negative controls (Yamaguchi et al. 1995). These findings were confirmed in a group of patients with Psoriatic Arthritis (Gladmann et al. 1986), however, B39 disease association was not detected in AS B27 negative patients from Caucasian and Mexican Mestizo origin (Maksymowych et al. 2000) and in another study performed in the UK (Brown et al. 1996). An association of AS with HLA-B*1403, an allele found exclusively in African or Afro – American populations, was recently reported in the population of Togo (Lopez-Larrea et al. 2002). In this population AS is a rare disease, but the investigation of eight AS patients identified B*1403 in four of them (50%). This allele has a B pocket similar to HLA-B27 and B-39 and its peptide specificity should be explored. It has been speculated that Class II alleles (DR1, DR2, DR7 and DR8) might also be associated with AS susceptibility. Data derived from genome-wide screening for susceptibility loci in AS extend the HLA allelic association to the MHC region between markers *D6S276* and *DRB1* (Brown et al. 1998). DR1 may possibly be a susceptibility marker for AS in some populations, and it was shown that DR4 contributes significantly to a genetic predisposition to SpA in the French population (Brown et al. 1998; Said-Nahal et al. 2002).

The Pathogenic Role of HLA-B27 in Spondyloarthropathies

The key problem associated with studying the pathogenesis of AS and SpA disorders is the analysis of a T-cell response in the absence of a known target antigen. There are several theories, that are not mutually exclusive, to explain the role of HLA-B27 in SpA pathogenesis.

The “Arthritogenic” Peptide Theory

The well documented association of acute reactive arthritis (ReA) with enteric bacterial

infection has led many researchers to look for similar associations with ankylosing spondylitis (AS). Hypothesis on disease mechanism have thus concentrated on putative unique HLA-B27 structural features (amongst HLA molecules) that would be shared with bacteria. Molecular mimicry between bacterial microorganisms and HLA-B27 has traditionally been viewed in terms of cross-reacting antibodies. Short sequences of HLA-B27 have been identified that are shared by arthritis-inducing bacteria and act as a target for these cross-reacting antibodies (Lopez de Castro 1998). Benjamin and Parham (1990) proposed the "arthritogenic peptide theory" which is based on the molecular mimicry model. It proposes the existence of a bacterial peptide that cross-reacts antigenically with a self protein found only in the joints. Cross reaction of some activated CTL with this self peptide would lead to autoimmune tissue damage and inflammation. Thus, the alleged arthritogenic peptide would be presented through HLA-B27 to CD8⁺ T cells, mediating an anti-self pathogenic response that would be propagated by further peptide released from damaged tissues. The fact that different HLA-B27 subtypes are associated with the disease has led to studies of the disease association and peptide-binding specificity of different subtypes.

Thymic Selection of T-Cell Repertoire

Thymic selection of T-cell repertoire is another theory explaining the association of HLA-B27 with SpA. According to the molecular mimicry, shared epitopes between the host and the bacteria would trigger autoimmunity if the immune system would elude the mechanism of tolerance (Lopez Larrea et al. 1996). The immune tolerance to self is established and maintained by the process called "clonal deletion". In the thymus, the autoreactive lymphocytes are eliminated by apoptosis during the development. However, there are antigens that are present exclusively in certain tissues and the T-cell specific for these antigens cannot undergo thymic selection. There are additional mechanisms that can inactivate these lymphocytes, although some T-cells can escape this process. Fortunately, no autoimmune response occurs because the self peptides are not appropriately presented to the autoreactive T-cells. However, in some circumstances, a bacterial antigen that mimics a self protein can activate the T-cells and

promote an autoimmune response.

Crossreactivity Between Antibodies Directed at Bacterial Proteins and HLA-B27

Another theory explaining the association of HLA-B27 with the SpA is based on the crossreactivity between antibodies directed at bacterial protein and HLA-B27. The arthritogenic peptide hypothesis suggests that an autopeptide derived from a polymorphic region of the HLA-B27 sequence with homology to proteins from arthritogenic bacteria would be naturally presented by HLA-B27. Thus, the T-cells that are not deleted in the thymus (CD4⁺ or CD8⁺ T-cells) are activated by the bacterial peptide in conjunction with the MHC class II molecules. The CD4 T-cells could activate the inflammatory T-helper 1 cells (Th1) which are implicated in the cellular immune response, and the T-helper 2 cells (Th2) which promote the production of antibacterial antibodies that cross-react with HLA-B27 or other self proteins (Lopez-Larrea et al. 1996). Although anti HLA antibodies are found in patients with SpA, controlled studies show no evidence that they are specific for HLA-B27 (Bowness et al. 1999).

HLA-B27-Derived Peptides are Presented by HLA Class II Molecules to T Cells

This theory suggests that peptides resulting from HLA-B27 molecule degradation may be presented as an auto antigen by class II HLA to CD4 T cells (Bowness et al. 1999). In fact class I derived peptides is some of the most prominent peptides eluted from HLA-DR molecules, and may show promiscuous binding to many HLA-DR alleles (Davenport 1995; Chiez et al. 1993). Further it was observed that, at least two molecules of DR, presented a region of the class I molecule that was both unusual in its sequence and unique to B27 (Davenport et al. 1995). The results of another study indicated that HLA-B27-derived peptides could be recognised as auto antigens by peripheral blood T lymphocytes of HLA-B27 positive AS patients and, to a lesser extent, B27 positive healthy controls (Marker-Hermann et al. 1997). This hypothesis was recently abandoned because data from transgenic mice demonstrated that MHC class II molecules are not required for the development of arthritis (Khare et al. 1998).

Altered-Self Hypothesis

This theory has been related mainly with the presence of an unpaired Cys 67 within the extracellular α_1 domain since this residue is present in the majority of B27 subtypes and not in most of the other HLA molecules. Three mechanisms were proposed to explain how Cys 67 could predispose to arthritis:

- Cys 67 might be chemically modified under certain conditions to alter the antigenicity of HLA-B27 (MacLean et al. 1992).
- A similar chemical modification might alter the peptide-binding properties of HLA-B27 (Gao et al. 1996).
- The presence of Cys 67 might be responsible for unusual features in the cell biology of HLA-B27, such as the ability to dimerise (Allen et al. 1999).

MacLean et al. (1992) proposed that Cys 67 can undergo oxidation after translation, so that the B27 epitope varies according to local oxidative conditions. This will alter the specificity of processed peptides, which can be accommodated by the presentation groove of B27, and could lead to self reactive immunological responses. This study provides evidence that the unpaired cysteine on most of B27 positive cell surfaces may have already undergone intracellular sulphhydryl interactions and this might provide an opportunity for the binding of particular pathogenic peptides, or for deleterious alterations of B27. They suggested that the unpaired sulphhydryl of most B27 cell surfaces is inaccessible as a result of previous covalent interactions, either inside the cell or shortly after movement to the cell membrane.

Autoimmunity in HLA-B27-positive patients could also be triggered by the molecule biochemical change. The highly reactive cysteine residue at position 67 could form disulfite bonding with thiol agents, causing a conformational change in B27 molecule, which may be recognised by CTLs as a true "altered self" (Benjamin and Parham 1990). Homocysteine is a major thiol in human plasma and is also a metabolic product of bacteria, such as *salmonella* and *yersinia*. The same authors confirmed this view, suggesting that B27-restricted Homocysteine-specific CTLs (Hom-CTL) might be the missing link between B27, AS and ReA. In fact Whelan and Archer (1993) have reported that the sulphhydryl group of B27 Cys 67 is much more

active than that of B38, B39 and B65. The sulphhydryl activity of an unpaired cysteine residue in a protein molecule depends on the surrounding amino acid residues. In B27, the chemical reactivity of Cys 67 might be altered by the proximity of Lys 70, a residue that is unique to HLA-B27. However, other factors have to be evolved in this model since homocysteinemia is a known risk factor for cardiovascular diseases, but has not been yet associated to spondyloarthropathies (Marker-Hermann and Hohler 1998)

Another potential pathogenic role of HLA-B27 Cys 67 is the capacity conferred to the HLA molecule to form heavy-chain homodimers not associated with β_2 microglobulin (β_2m) light-chains (Khare et al. 1996). A murine model have been developed in which HLA-B27 transgenic mice lacking β_2m develop arthritis (MacLean et al. 1992). The authors verified that disease incidence was reduced when the same animals were treated with HC10, a monoclonal antibody that recognises the heavy (H) chain of HLA-B27. Allen et al (1999) work shows that HLA-B27 can form a cys 67-dependent heavy chain homodimer (HC-B27) in the absence of β_2m , and these complexes are recognised by the conformation specific Ab W6/32. HC-B27 formation is dependent on disulfite bonding through Cys 67. Such bonding would almost certainly require unwinding of the α_1 helix around Arg 62; this is consistent with HC-B27 acquisition of HC10 (H chain specific mAb) epitope. The acquisition of the HC10 epitope and the lost ME1 antibody reactivity suggests a structural change in the region surrounding Cys 67. Such change could open the groove to accommodate longer peptides permitting the binding of an altered repertoire of antigenic peptides, which might be longer at their N-terminal compared with most peptide bound to MHC class I chains. In addition, the unwounded α_1 helix might resemble the α chain helix of an MHC class II molecule, possibly recognised by CD4 T cells. Though, a possible mechanism by which expression of HC-B27 could lead to joint inflammation is by the presentation of peptides to either CD4 or CD8 cells chains. Alternatively, HC-B27 might be a ligand for NK receptors or other receptors, or it might be a target of an antibody response (Lanier 1998).

HLA-B27 Misfolding

HLA-B27 unusual tendency to misfold is due to its B pocket and is associated with the

formation of high molecular disulfide-bond complexes and with the increased retention of HLA-B27 heavy chains in the ER (Colbert et al. 2000). HLA-B27 misfolding, under conditions of up-regulation in heavy-chain synthesis, could activate or modulate ER stress signaling pathways, and such effect could underline the ability of B27 to cause disease (Colbert 2000a, b; Colbert and Prahalad 2001). The slow-folding kinetics and misfolding is an interesting biochemical feature of HLA-B27 that must be further investigated. However, this hypothesis is not based on largely sufficient evidence and some critical aspects remain to be explained. If HLA-B27 misfolding, rather than antigen presentation, is a key to the pathogenesis of SpA the mechanism involved should explain the specificity of misfolding for HLA-B27; the different behaviour of the several subtypes associated with AS; and the way in which arthritogenic bacteria co-operate with or exacerbate HLA-B27 misfolding (Ramos and Lopez de Castro 2002).

CONCLUSIONS

The strong association between HLA-B27 and SpA is known for several decades however, the exact mechanism by which this allele is involved is still unknown. Other associations of AS with genes within the MHC or in other chromosomes were already described (Brown et al. 2000), and genome-wide scans identified several regions (chromosomes 2, 10, 16 and 19) with LOD scores significant for linkage (Brown et al. 1998). The recent identification of additional genes involved in the pathogenesis of the disease suggests that genome-wide scans and experimental animal models are essential for a better understanding of the factors involved in the development of SpA.

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REFERENCES

- Allen RL, O'Callaghan CA, Mc Michael AJ, Bowness P 1999. Cutting edge: HLA-B27 can form a novel beta 2-microglobulin-free heavy chain homodimer structure. *J Immunol*, **162**: 5045-48.
- Alvarez I, Lopez de Castro JA 1999. HLA-B27 and immunogenetics of spondyloarthropathies. *Curr Opin Rheumatol*, **12**: 248-253.
- Armas JB, Gonzalez S, Martinez-Borra et al. 1999. Susceptibility to ankylosing spondylitis is independent of the Bw4 and Bw6 epitopes HLA-B27 alleles. *Tissue Antigens*, **53**: 237-243.
- Balas A, Santos S, Garcia-Sanchez F et al. 1998. Complete coding sequence of HLA-B2712: a serologic B27-negative antigen associated to Bw6. *Tissue Antigens*, **51**: 394-7.
- Benjamin R, Parham P 1990. Guilt by association: HLA-B27 and Ankylosing Spondylitis. *Immunol Today*, **11**: 137-42 .
- Bowness P, Zaccai N, Bird L, Jones Y 1999. HLA-B27 and disease pathogenesis: new structural and functional insights. *Expert Reviews Mol Med*, 1-10.
- Brewerton DA, Caffrey M, Hart FD et al. 1974. Ankylosing spondylitis and HLA-B27. *Lancet*, **1**: 904 - 907.
- Brown M, Jepson A, Young A et al. 1997. Spodyloarthritis in West Africa : Evidence for a non-B27 protective effect. *Ann Rheum Dis*, **56**: 68-70.
- Brown MA, Pile KD, Kennedy LG et al. 1996. HLA class I association of ankylosing spondylitis in a white population in The United Kingdom. *Ann Rheum Dis*, **55**: 268-70.
- Brown MA, Bunce MA et al. 1996. HLA-B associations of B27 negative ankylosing spondylitis: commentary. *Arthritis Rheum*, **39**: 1768-9.
- Brown MA, Pile KD, Kennedy LG et al. 1998. A genome wide-screen for susceptibility loci in ankylosing spondylitis. *Arthritis Rheum*, **41**: 588-95.
- Brown MA, Kennedy LG, Darke C et al. 1998. The effect of HLA-DR genes on susceptibility to and severity of ankylosing.spondylitis. *Arthritis Rheum*, **41**: 460-5.
- Brown MA, Edwards S, Hoyle E et al. 2000. Polymorphisms of the CYP2D6 gene increase susceptibility to ankylosing spondylitis. *Human Mol Genet*, **9**: 1563-1566.
- Calin A 1998a. Terminology, introduction, diagnostic criteria, and overview. In: A Calin, JD Taurog (Eds.): *The Spondylarthritides*. Oxford: Oxford University Press. Pp 1-26.
- Calin A 1998b. Reactive arthritis and Reiter's syndrome – the clinical spectrum. In: A Calin, JD Taurog (Eds.): *The Spondylarthritides*. Oxford: Oxford University Press. Pp 41-57.
- Chicz RM, Urban RG, Gorga JC et al. 1993. Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles. *J Exp Med*, **178**: 27-47
- Colbert RA, Dangoria NS, Mear JP 2000. Mechanism and consequences of HLA-B27 misfolding. *Arthritis Rheum*, **43**: S399.
- Colbert RA 2000a. HLA-B27 misfolding: a solution to the spondyloarthropaty conundrum? *Mol Med Today*, **6**: 224-230;
- Colbert RA 2000b. HLA-B27 misfolding and spondyloarthropathies: not so groovy after all? *J Rheumatol*, **27**: 1107-1109.
- Colbert RA, Prahalad S 2001. Predisposing factors in the spondyloarthropathies: New insights into the role of HLA-B27. *Curr Rheumatol Rep*, **3**: 401-411.
- D'Amato M, Fiorillo MT, Carcassi C et al. 1995.

- Relevance of residue 116 of HLA-B27 in determining susceptibility to ankylosing spondylitis. *Eur J Immuno*, **25**: 3199-201.
- Darke C, Street J, Hammond L et al. 2002. Immunogenetic study of a new HLA allele, B*2723. *Tissue Antigens*, **60**: 400-3.
- Davenport MP 1995. The promiscuous B27 hypothesis. *Lancet*, **346**: 500-1.
- Davenport MP, Quinn CL, Chicz RM et al. 1995. Naturally processed peptides from two disease-resistance associated HLA-DR-13 show related sequence motifs and the effects of the dimorphism at position 86 of HLA-DR b chain. *Proc Natl Acad Sci USA*, **92**: 6567-71.
- Feldman D, Kearns J, Wu J 2002. Identification and sequencing of HLA-B*0714 and B*2718 alleles and novel exon 1 sequences of B*0709 and B*2714 alleles in potential bone marrow donors. *Tissue Antigens*, **59**: 426-9.
- Feltkamp TE 1996. Non HLA-B27 genetic factors in HLA-B27 associated disease. *Clin Rheumatol*, **15** (Supp 11): 40-43.
- Gao, XM, Wordsworth P, Mc Michael AJ et al. 1996. Homocysteine modification of HLA antigens and its immunological consequences. *Eur J Immunol*, **26**: 1443-50.
- Gans CP, Mitton W, Ng J, Hartzman RJ et al. 2002. Seven new HLA-B alleles associated with antigens in the B7 CREG. *Tissue Antigens*, **59**: 229-31.
- Ghosh K, Shankarkumar U, Shetty S, Mohanty D 2003. Chronic synovitis and HLA B27 in patients with severe haemophilia. *Lancet*, **361**: 933-934.
- Gladman DD, Anhorn KAB, Schachter RK, Mervat H 1986. HLA antigens in psoriatic arthritis. *J Rheumatol*, **13**: 586-92.
- Gofton JP 1980. Epidemiology, tissue type antigen and Bechterew's syndrome (ankylosing spondylitis) in various ethnical populations. *Scand J Rheum*, **9**: 166-168.
- Gonzalez-Roces S, Alvarez MV, Gonzalez S et al. 1997. HLA-B27 polymorphism and worldwide susceptibility to ankylosing spondylitis. *Tissue Antigens*, **49**: 116-123.
- Gonzalez-Roces S, Brautbar C, Peña M et al. 1994. Molecular analysis of HLA-B27 haplotypes in Caucasoids. Frequencies of B27-Cw in Jewish and Spanish populations. *Hum Immunol*, **41**: 127-134.
- Gonzalez S, Garcia-Fernandez S, Martinez-Borra J et al. 2002. High variability of HLA-B27 alleles in ankylosing spondylitis in the population of northern Spain. *Hum Immunol*, **63**: 673 - 6.
- Hasegawa T, Sugahara Y, Moryiama Y et al. 1996. A new HLA-B27 allele found in a healthy Japanese. *Hum Immunol*, **47** - 8.
- Hill AV, Allsopp CE, Kwiatkowski D et al. 1991. HLA class I typing by PCR: HLA-B27 and an African B27 subtype. *Lancet*, **337** (8742): 640-42.
- Jardetzky TS, Lane WS, Robinson RA et al. 1991. Identification of self peptides bond to purified HLA-B27. *Nature*, **353**: 326 - 329.
- Khan MA 1995. HLA-B27 and its subtypes in world populations. *Curr Opin Rheumatol*, **7**: 263-269.
- Khan MA 1998a. Ankylosing spondylitis: clinical aspects. In: A Calin, JD Taurog (Eds.): *The Spondylarthritides*. Oxford: Oxford University Press. Pp 27- 40.
- Khan MA 1998b. A worldwide overview : the epidemiology of HLA-B27 and associated spondyloarthritides. In: A Calin, JD Taurog (Eds.): *The Spondylarthritides*. Oxford: Oxford University Press. Pp 17-26.
- Khare SD, Bull MJ, Hanson J et al. 1998. Spontaneous inflammatory disease in HLA-B27 transgenic mice is independent of MHC class II molecules: a direct role for B27 heavy-chains and not B27-derived peptides. *J Immunol*, **160**: 101-6.
- Khare SD, Hansen J, Luthra HS, David CS 1996. HLA-B27 Heavy chains contribute to spontaneous inflammatory disease in B27/human beta 2-microglobulin (beta2m) double transgenic mice with disrupted mouse beta2m. *J Clin Invest*, **98**: 2746-55.
- Klein J, Sato A 2000. The HLA system – first of two parts. *N Eng J Med*, **343** (10): 702-709.
- Kim MA, Kang SJ, Han KS et al. 2000. Five new HLA alleles (A*0241, B*2720, B*3531, DRB1*1339) found in Koreans. *Hum Immunol*, **61S**: 50-50.
- Lamas JR, Paradela A, Roncal F et al. 1999. Modulation at multiple anchor positions of the peptide specificity of HLA-B27 subtypes differentially associated with ankylosing spondylitis. *Arthritis Rheum*, **42**: 1975-1985.
- Lanier LL 1998. Follow the leader: NK cell receptors for classical and nonclassical MHC class I. *Cell*, **92**: 705-7.
- Lopez de Castro JA 1989. HLA-B27 and HLA-A2 subtypes: structure, evolution and function. *Immunol Today*, **10** (7): 239-246.
- López de Castro J 1998. The pathogenic role of HLA-B27 in chronic arthritis. *Curr Opin Immunol*, **10**: 59-66.
- Lopez-Larrea C, Sujirachato K, Mehra NK et al. 1995. HLA-B27 subtypes in Asian patients with ankylosing spondylitis. Evidence for new associations. *Tissue Antigens*, **45**: 169-76.
- Lopez-Larrea C, Mijiyawa M, Gonzalez S et al. 2002. Association of Ankylosing Spondylitis with HLA B*1403 in a West African population. *Arthritis Rheum*, **46**: 2968 –71.
- Lopez-Larrea C, Gonzalez-Roces S, Alvarez V 1996. HLA-B27 structure, function, and disease association. *Curr Opin Rheumatol*, **8**: 296-308.
- López-Larrea C, González S, Martínez-Borra J 1998. The role of HLA-B27 polymorphism and molecular mimicry in spondyloarthropathy. *Mol Med Today*, 540-549.
- MacLean L, Macey M, Lowdell M et al. 1992. Sulphydryl reactivity of the HLA-B27 epitope: accessibility of the free cysteine studied by flow cytometry. *Ann Rheum Dis*, **51**: 456-60.
- MacLean I 1992. HLA-B27 subtypes: implications for the spondyloarthropathies. *Ann Rheum Dis*, **51**: 929-931
- Maksymowych WP, Tao S, Vaile J et al. 2000. LMP2 polymorphism is associated with extraspinal disease in HLA-B27 negative Caucasian and Mexican Mestizo patients with ankylosing spondylitis. *J Rheumatol*, **27**: 183-189.
- Märker-Hermann E, Büschenfelde K-H M, Wildner G 1997. HLA-B27-derived peptides as autoantigens

- for T lymphocytes in ankylosing spondylitis. *Arthritis Rheum*, **40**: 2047-54.
- Märker-Hermann E, Höhler T 1998. Pathogenesis of human leucocyte antigen B27-positive arthritis. *Rheum Dis Clin North Am*, **24**: 865-81.
- Martinez-Borra, Gonzalez S, Lopez-Vasquez et al. 2000. HLA-B27 alone rather than B27-related class I haplotypes contributes to ankylosing spondylitis susceptibility. *Hum Immunol*, **61**: 131 – 9.
- Nasution AR, Mardjuadi A, Kunmartini S 1997. HLA-B27 subtypes positively and negatively associated with spondyloarthopathy. *J Rheumatol*, **24**: 1111-1114.
- Ozgul A Orkunoglu FE, Sengul A et al. 2002. HLA-B27 subtypes in Turkish patients with spondyloarthropathy. *Clin Exp Rheumatol*, **20** (4): P2.5,
- Ramos M, Lopez de Castro JA 2002. HLA-B27 and the pathogenesis of spondyloarthritis. *Tissue Antigens*, **60**: 191-193.
- Ren EC, Koh WH, Sim D et al. 1997. Possible protective role of HLA-B2706 for ankylosing spondylitis. *Tissue Antigens*, **49**: 67-69.
- Reveille JD, Suarez AM, Russell AS et al. 1994. HLA in ankylosing spondylitis: is HLA-B27 the only MHC gene involved in disease pathogenesis? *Sem Arthritis Rheum*, **23**: 295-309.
- Robinson WP, van der Linden SM, Khan MA et al. 1989. HLA-Bw60 increases susceptibility to ankylosing spondylitis in HLA-B27+ patients. *Arthritis Rheum*, **32**: 1135 – 41.
- Said-Nahal R, Miceli-Richard C, Gautreau C et al. 2002. The role of HLA genes in familial spondyloarthropathy: a comprehensive study of 70 multiplex families. *Ann Rheum Dis*, **61**: 201-206.
- Shankarkumar U, Ghosh K, Mohanty D 2002. HLA-B27 polymorphism in Western India. *Tissue Antigens*, **60**: 98-101.
- Shankarkumar U, Ghosh K, Mohanty D 2003. Novel HLA B*2714 and B*2708 allele associations in seronegative spondylarthritis patients and Haemophilia patients with chronic synovitis in India. *Tissue Antigens*, **62**: 175-178.
- Schlosstein L, Terasaki P, Bluestone R et al. 1973. High association of an HLA antigen, w 27, with ankylosing spondylitis. *N Eng J Med*, **288**: 704 - 706.
- Steiner NK, Gans C, Baldassarre L et al. 2003. Twenty-five novel HLA-B alleles. *Tissue Antigens*, **62**: 263-6.
- Steiner NK, Jones P, Kosman C et al. 2001. Novel HLA-B alleles associated with antigens in the 7C CREG. *Tissue Antigens*, **57**: 486-8.
- Tamouza R, Mansour I, Bouguacha N et al. 2001. New HLA-B*27 allele (B*2719) identified in a Lebanese patient affected with ankylosing spondylitis. *Tissue Antigens*, **58**: 30-3.
- Voorter CEM, Swelsen WTN, van den Berg-Loonen EM 2002. B*27 in molecular diagnostics: impact of new alleles and polymorphism outside exons 2 and 3. *Tissue Antigens*, **60**: 25-35.
- Wordsworth P, Brown MA 1998. *HLA-B27, ankylosing spondylitis, and the spondyloarthropathies*. In Calin A, Taugrog JD, (eds): *The Spondylarthritides*; Oxford: Oxford University Press. 179-193.
- Whelan MA, Archer JR 1993. Chemical reactivity of an HLA-B27 thiol group. *Eur J Immunol*, **23**: 3278-85.
- Yamaguchi A, Tsuchiya N, Mitsui H et al. 1995. Association of HLA-B39 with HLA-B27 negative ankylosing spondylitis and pauciarticular juvenile rheumatoid arthritis in Japanese patients. Evidence for a role of the peptide-anchoring B pocket. *Arthritis Rheum*, **38**: 1672-7.