HLA-B27

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Contents

Abstract

Possession of the Human Leukocyte Antigen (HLA) class I molecule B27 is strongly associated with Ankylosing Spondylitis (AS), but its pathogenic role is unknown. Two broad theories most likely explain the role of HLA-B27 in AS pathogenesis. The first is based on the natural immunological function of HLA-B27 to present antigenic peptides to cytotoxic T cells. Thus HLA-B27-restricted immune responses to self antigens or “arthritogenic” peptides might drive immunopathology.

B27 can also “behave badly”. Thus B27 can misfold during assembly, leading to ER stress and autophagy responses. β2m-free B27 heavy chain (FHC) structures including homodimers ($B27_2$) can also be expressed at the cell

surface following endosomal recycling of cell-surface heterotrimers. Cell surface FHC and $B27_2$ bind to innate immune receptors on T, NK and myeloid cells with pro-inflammatory effects.

This review will describe the natural function of HLA-B27, its disease associations and the current theories as to its pathogenic role.

Introduction

Possession of HLA-B27 is very closely linked with the development of a group of common inflammatory rheumatic diseases known as the Spondyloarthritides (SpA), of which Ankylosing Spondylitis (AS) is the prototype, which collectively affect over 0.5% of the population.

This review will describe the natural function of HLA-B27, its disease associations and the current theories as to its pathogenic role - which despite intense investigation remains unknown. The current theories are briefly summarized in **Figure 1**.

1. The natural functions of HLA-B27

Human Leukocyte Antigens (HLA), encoded on chromosome 6, make up the human Major Histocompatibility Complex and are the most polymorphic proteins known. The classical class 1 A B and C molecules are expressed on the cell surface of almost all nucleated cells. The B27 allomorph is one of the commonest B alleles in Caucasian populations. The principle natural function

of HLA-B27 is to present endogenous (i.e. intracellular) peptides to T lymphocytes, predominantly to the T cell Receptor for Antigen (TCR) of cytotoxic T cells.

1.1 Peptide presentation by HLA-B27 to CD8+ Cytotoxic T cells

HLA-B27 heavy chains are synthesized in the endoplasmic reticulum where they form part of a multi-protein complex known as the peptide loading complex. Following the original observation of MHC restriction by Zinkernagel and Doherty(1),(2), Townsend and McMichael, studying recognition of influenza-infected cells by cytotoxic T cells, showed firstly that the primary antigenic target was the internal nucleoprotein (rather than the expected surface haemaglutinin) and secondly that this target was a relatively short peptide(3). Definition of HLA-B27-restricted epitopes from HIV(4) and Influenza(5) suggested common structural features but could not distinguish between those required for B27 binding and those affecting T cell recognition. Subsequently Madden and colleagues solved the crystal structure of HLA-B27 (the second to be determined after that of HLA-A2). This structure, shown in **Figure 2**, revealed a peptide binding groove with a “B” pocket (comprising different residues to that of HLA-A2) perfectly binding the positively charged side chain of an arginine residue at the second position of bound peptide(6),(7). B27 residues including a glutamic acid residue at position 45 and a cysteine at 67 contributed to the B pocket specificity. At the same time peptide elution from a human B27 homozygous B lymphoblastoid cell line not only confirmed that all peptides carried an arginine at the second residue from

the N terminus, but also showed that many thousands of different peptides were bound to the B27 molecules expressed at the cell surface, and that these were most commonly derived from self-proteins, including HLA molecules(8). Identification of additional naturally processed and presented viral epitopes(9) confirmed that immunodominant B27-binding epitopes all possess arginine as their second residue. Subsequent studies using synthetic peptides have shown that peptides with non-arginine P2 (including glutamine, methionine and post-synthetically modified residues(10)) are capable of binding to HLA-B27, but the physiological importance of this observation is not known.

HLA-B27 heavy chains form heterotrimeric complexes with β2m and intracellular peptides within the endoplasmic reticulum (ER). Within the ER HLA heavy chains are part of the multimolecular peptide-loading complex that includes calnexin, calreticulin, tapasin, and the thiol oxidoreductase ERp57. The pathways of antigen processing are reviewed by Blum, Wearsch and Cresswell(11). This complex facilitates correct MHC folding, β2m binding and peptide loading. Tapasin is thought to play a key role in optimisation of the cargo of bound peptides, although HLA-B27 is less dependent on tapasin than other alleles such as B*4402(12). These heterotrimeric complexes (henceforth called HLA-B27) egress to the cell surface where they are recognized by CD8+ cytotoxic T cells.

HLA-B27-restricted CTL responses to viruses are often tightly focussed resulting in immunodominant responses to small numbers of epitopes. In HIV infection viral mutation leading to loss of CTL recognition is consistently associated with disease progression, providing strong evidence for a key role of CTL in viral control(13). Epidemiological studies have also supported the concept that HLA-B27 confers protection against viral infections, indeed giving a prognostic advantage following both HIV(14) and hepatitis C infection, in the latter case escape from CD8-mediated CTL is limited by loss of viral fitness and T cell cross-reactivity(15). Thus HLA-B27 may act as a "double-edged sword", simultaneously enhancing anti-viral immunity and predisposing to SpA(16)[6]. It should be pointed out that such a property could result either directly from its peptide-binding properties or indirectly from "adjuvant"-like effects detailed in sections 3.2 and 3.3.

Over 100 subtypes of HLA-B27 are now recognized, which differ in primary amino acid sequence whilst sharing key structural, peptide binding and antigenic features. This is discussed in section 2.2. The underlying (and unproven) assumption is that this huge HLA polymorphism is driven by the need during infection for diversity in peptide binding and presentation to T cells.

1.2 Peptide presentation by HLA-B27 to NK cells

Natural killer cells use specific receptors for HLA recognition, such as the Killer Immunoglobulin–like Receptors (KIR). Shortly after the discovery that

the cognate KIR for B27 recognition is KIR3DL1(17), came the appreciation that this recognition was sensitive to the nature of peptide bound to the B27 molecule(18). These key functional studies were subsequently confirmed using peptide substitutions guided by crystal structures. Thus B27 complexed with the immunodominant EBV epitope (EBNA3C 258-266 RRIYDLIEL) does not bind to KIR3DL1, however binding occurs if the P8 glutamate is substituted to threonine(19). Rather than selectively or specifically recognizing a given peptide, it is likely that KIR3DL1 recognition of HLA-B27 will occur unless there is steric inhibition, with KIR3DL1 recognition of B27 exquisitely sensitive to the nature of the P8 amino acid side chain. The Leucocyte Ig-like Receptors LILRB1 and LILRB2 both recognize HLA-B27/peptide complexes(20) but evidence for peptide specificity has been conflicting(21).

1.3 Atypical peptide presentation by HLA-B27 – e.g. to CD4 T cells

There is evidence that HLA-B27 can interact in non-canonical ways with both peptide and T cells. Thus B27 is capable of binding peptides significantly longer than the usual 9-12 amino acids. These complexes are recognized by the MARB4 antibody(22), which also detects a population of beta 2 microglobulin-free peptide-containing B27 complexes at the cell surface(23). Atypical peptide presentation by HLA-B27 also occurs– thus Boyle and colleagues demonstrated that human CD4 T cells can recognize HLA-B27(24). This was confirmed in a human TCR-transgenic HLA-B27 positive murine model(25).

Another possibility is that the peptide binding groove of B27 might accommodate a small molecule (perhaps a gut microorganism metabolite) that consequently alters the B27 repertoire similar to the effect of the anti-retroviral drug abacavir on HLA-B*5701(26). This could then result in “neoimmune” responses to novel antigens or a heightened multi-specific immune response akin to alloreactive immune responses.

1.4 Other functions of HLA-B27 heterotrimers and free heavy chains

HLA molecules play a central role in thymic selection of the T cell repertoire, and therefore individuals carrying the HLA-B27 allotype will almost certainly harbour both potentially autoreactive TCR specificities and also “holes” in their TCR repertoire, either or both of which could be important in SpA pathogenesis.

HLA class molecules, and in particular beta-2 microglobulin-free heavy chains, are expressed on activated and transformed lymphoblastoid cells(27). Furthermore HLA class 1 heavy chains can also be secreted extracellularly under these circumstances(28). These findings suggest that changes in HLA heavy chain expression may be indicative of cellular status or activation - however relatively little is known of their sensing and downstream effects in cis or trans.

HLA class 1 molecules have also been shown to have a role in both neurodevelopment (29) and mate selection(30). It is thus possible that HLA-B27 may have a selective developmental or reproductive advantage that maintains its prevalence in multiple human populations despite the almost certain evolutionary disadvantages associated with increased SpA prevalence. Unexpectedly the prevalence of HLA-B27 has been found to fall with age, suggesting that possession on B27 has a survival disadvantage after middle age(31). This is an intriguing finding that requires replication in different populations. Although no explanation for these findings has been found, one testable hypothesis is that B27-positive individuals have slightly higher levels of chronic inflammation predisposing to a variety of ischaemic and neoplastic diseases.

Another possibility is that HLA-B27 might have interactions with gut microbiota. For example HLA-B27 might act as a receptor for bacterial dissemination, or microorganisms or their metabolic products might have effects on peptide binding (as described in 1.3).

2. HLA-B27 and Spondyloarthritis

Possession of HLA-B27 is very closely linked with the development of a group of common inflammatory rheumatic disease collectively called the Spondyloarthritides (SpA), which are listed in Table 1. Ankylosing Spondylitis (AS) is the prototypic SpA, affecting approximately 0.4% of the population(31). Collectively the SpA may affect approximately 0.8% of the population. Whilst

roughly 94% of AS patients are HLA-B27 positive, and whilst B27 is the most important genetic factor, it still only contributes about 1/3 of the total heritability of AS (which is remarkably high at approximately 90%). Currently over 40 other contributory genetic loci have been identified, including genes involved in type 17 immune responses and antigen processing(32),(33), but many more remain to be identified and their roles elucidated.

2.1. HLA-B27 disease associations

Caffrey and James, and Shlosstein and colleagues independently described the association of Ankylosing Spondylitis with the HLA allele A27, as it was then designated, in 1973(34),(35). Disease association with Reactive Arthritis was reported shortly afterwards(36). The separation of the "seronegative" arthritides from rheumatoid arthritis (usually but not invariably rheumatoid factor "seropositive") had only been made in the 1950s. Subsequently the appreciation that the Spondyloarthritides (or spondyloarthropathies) are a group of diseases sharing key clinical features and genetic factors (primarily but not only HLA-B27) has proved useful in progressing our understanding of pathogenesis and treatment. Thus for example not only do at least 25% of patients with AS develop anterior uveitis (inflammation of the anterior chamber of the eye) over their lifetime(37), but this condition can occur without concomitant AS and as such is still associated with B27(38). The different SpA are briefly described in Table 1 together with their HLA-B27 associations. Ankylosing Spondylitis is the commonest and most strongly B27-associated of the SpA. Over 90% of Caucasian AS patients are HLA-B27 positive, with an Odds ratio of 171(39). The prevalence of AS in different

populations broadly reflects the prevalence of HLA-B27. Thus approximately 9% of UK Caucasians are HLA-B27 positive and 0.4% have AS. In northern Scandinavia and northern Native American and Canadian populations the prevalence of both B27 and SpA are higher, whereas in sub-Saharan Africa B27 is rare and so is AS. The influence of different HLA-B27 subtypes is discussed in section 2.2.

2.2 HLA-B27 subtypes and AS

Over 100 subtypes of HLA-B27 are currently recognized and are designated HLA-B*2701 to HLA-B*27106, defined by their DNA sequence. B*2705, by far the commonest subtype in Caucasian populations, is thought to be the ancestral subtype. All B27 subtypes share common amino acid residues within the B pocket (including E45 and C67) and a very strong preference for arginine at the second position of bound peptide. The relative prevalence of the different common B27 subtypes is summarized in **table 2:** and is reviewed by Kahn(40). The common subtypes in Caucasian populations are B*2705 and B*2702. In Asian populations B*2704 and B*2706 predominate. B*2704 is definitely associated with AS(41) but B*2706 is weakly or not associated(42). B*2709, a rare subtype found almost exclusively in people of Sardinian descent, has been reported to be weakly or not associated with AS(43), although given that it is found on an extended haplotype on chromosome 6 containing alleles of many other immunologically important genes, linkage to protective alleles cannot be completely discounted. Notably patients with AS who carry B*2709 have been described, although even here

the carriage of this allele on the “second” haplotype (i.e. that the allotype is a passenger rather than a disease driver) cannot of course be excluded.

B*2709 differs from HLA-B*2705 only at a single residue, position 114, although this appears to be sufficient to subtly alter the repertoire of peptides bound ((44) and see section 3.1). B*2706, also weakly or not associated with AS, differs from the strongly disease-associated B*2704 only at positions 114 and 116 and from B*2705 at a single further residue 152. Positions 114 and 116 lie at the base of the peptide-binding groove and contribute to the F pocket. These amino acid substitutions have been shown to influence the nature of the amino acid side chain at the C terminus, although the overall peptide repertoires show considerable overlap(45). Interestingly the aspartic acid residue at P116 of B*2705 has been shown to variably form salt bridge with the P5 residue of bound peptide, with B*2705 capable of binding a single peptide in two conformations(46).

3. HLA-B27 pathogenic mechanisms

The mechanism by which HLA-B27 causes disease is not known. Here the three currently most favoured hypotheses will be discussed in detail, together with the role of the aminopeptidase ERAP1, which is co-implicated in pathogenesis by genetic studies. HLA-B27 may present “arthritogenic” peptides to CD8 T cells, but B27 can also adopt aberrant forms both in the endoplasmic reticulum and at the cell surface, both of which can stimulate inflammation.

3.1 Presentation of “arthritogenic” peptides

Following the discovery that the immunological function of MHC class 1 molecules is to bind peptides derived from intracellular proteins (described in section 1.1), Parham and Benjamin proposed that Spondyloarthritis might be caused by HLA-B27 binding a peptide derived from a microorganism and eliciting a CD8 T cell response cross-reactive with a B27/self peptide combination. This process we now frequently call molecular mimicry, although somewhat confusingly the term molecular mimicry had previously been coined in the context of B27 to describe a process whereby a pathogenic factor “modified” B27 to make it antigenic.

A number of lines of evidence, summarized in table 3, support the arthritogenic peptide hypothesis. Firstly HLA-B27-restricted CD8 T cell responses have been identified specific for Salmonella or Chlamydia in patients developing reactive arthritis following these infectious triggers(47),(48). The latter were detected using tetrameric HLA-B27/peptide complexes in the affected joints, but at low frequency(48). Fiorillo and colleagues identified cross-reactive CD8 T cell responses in patients with SpA that recognized both an epitope from Epstein Barr virus and a self peptide derived from the vasoactive intestinal protein receptor VIPR(49). However it should be pointed out that EBV is not recognized as a trigger for SpA and so the implications of this finding are uncertain. Thus whilst cross-reactive and potentially arthritogenic CD8 responses undoubtedly occur, those identified are not consistently or temporally related to disease. Nevertheless it is entirely plausible that T cell responses to as yet undefined antigens play a

significant role in SpA pathogenesis, particularly given that in these diseases, unlike for example autoimmune thyroid disease, there is not a readily apparent autoantigenic target tissue. Indeed oligoclonal T cell expansions have been detected in the joints of SpA patients (50),(51),(52). Determining the specificity of these responses will be important given that a clear autoantigen has not been identified in SpA. New technologies to determine T cell specificities in an unbiased manner will likely assist in this search. One intriguing possibility is that B27-restricted responses may be driven by intestinal microflora.

The differential association of different HLA-B27 subtypes, which differ only in residues of their peptide-binding groove, broadly supports arthritogenic peptide mechanisms of disease causation. The non-disease-associated HLA-B*2709 subtype differs from *2705 only at position 114, where it carries histidine as opposed to aspartic acid(43). This residue in the floor of the peptide-binding groove contributes to the F pocket and this change appears to be sufficient to subtly alter the repertoire of peptides bound(44) residue. Thus peptides eluted from B*2709 generally contained hydrophobic C termini and did not accommodate tyrosine at this position(44).

The strong genetic association of AS with Endoplasmic Reticulum Aminopeptidase 1 (hereafter abbreviated to ERAP1), strongly suggests that ERAP1 most likely acts directly on the function of HLA-B27 within the antigen processing and presentation pathway. This is discussed in detail in section 3.5. Whilst at first sight this association would seem to favour the

arthritogenic peptide hypothesis, it would also be compatible with the intracellular misfolding and cell surface free heavy chain hypotheses discussed in sections 3.2 and 3.3.

As well as the difficulty demonstrating pathogenic CTL responses to arthritogenic peptides, studies using animal models have generally not supported this model. Thus CD8 T cells do not appeared to be required for disease in HLA-B27 transgenic rat models. This has been demonstrated by both antibody-mediated depletion(53) and CD8 alpha knockout(54).

Recently Sherlock and colleagues have elegantly demonstrated that IL23 (expressed using mini-circle technology) is sufficient alone to drive murine SpA-like disease by acting on entheseal resident CD3+ CD4-CD8- ROR gamma T-expressing T cells(55). These data would suggest that B27 need only act at the site of IL23 production to have its pathogenic effect and would argue against an arthritogenic peptide model.

3.2 HLA-B27, the unfolded protein response and the ER stress response

Shortly after demonstration in 1999 of the ability of HLA-B27 to form homodimers(56), Mear and colleagues demonstrated that HLA-B27 misfolds in the endoplasmic reticulum (with subsequent cytosolic degradation)(57). This misfolding phenotype could be corrected by replacing key B27 B pocket residues with those from HLA-A2, i.e. H9F (F for H at position 9), T24A,

E45M, I66K, C67V, and H70K. Notably the ability to form homodimers is not unique to HLA-B27. Homodimerisation can be induced in other class 1 molecules by slowing the rate of egress from the ER (e.g. by culture at 26 degrees C) and can be mediated by cysteines other than cys 67 including cys 164(58).

HLA-B27 transgenic rat bone marrow-derived macrophages (but not splenocytes) show evidence of HLA-B27 misfolding after cytokine stimulation and this correlates with augmented production of IL23(59),(60). HLA-B27 transgenic rats exhibit functional alterations in a number of cell populations which might correlate with misfolding, with both defects in dendritic cell populations and function(61) and enhanced ability to form osteoclasts described(62).

Appreciation of the molecular mechanisms underlying these changes has come from discovery that activation of the ER stress-induced transcription factor C/EBP homologous protein CHOP can lead to IL23 expression in dendritic cells(63). Interestingly enhanced Salmonella replication has been described within HLA-B27-transfected U937 monocytic cells, possibly mediated through effects of B27 heavy chains on the RNA-stabilizing protein Human antigen R(64).

Whilst these data (reviewed by Colbert and colleagues(65)), demonstrate that HLA-B27 is capable of driving an inflammatory ER stress response, alternate lines of evidence have argued against this mechanism as an important

pathological mechanism in driving Spondyloarthritis. Thus in the B27 transgenic rat model, introduction of additional copies of the human beta 2 microglobulin gene reduced misfolding (and colitis) whilst increasing the incidence and severity of arthritis(66). Studies of human tissue from AS patients have not convincingly shown evidence for UPR or ER stress in association with inflammation(67) (68), with the exception of one study showing upregulation of GRP78 in the peripheral joints of AS patients(69). Interestingly the study of Ciccia and colleagues provided evidence implicating autophagy in the gastrointestinal tract of AS patients(68). They studied immunohistochemical and gene expression intestinal biopsies from patients with AS and saw upregulation of autophagy-associated factors LC3II, ATG5 and ATG12. They also found that autophagy but not UPR was necessary for enhanced IL23 expression by gut-derived AS mononuclear cells(68).

Thus B27 is capable of ER misfolding and stimulation of ER stress and unfolded protein responses in vitro and in animal models. However at the current time there is relatively little direct evidence of this in human Spondyloarthritis. Further investigation of the interaction of HLA B27 with the autophagy pathway is clearly also warranted

3.3 Cell surface expression of free heavy chain forms of HLA-B27 heavy chain including homodimers, and their recognition by NK family immunoreceptors

The ability of HLA-B27 to form β2m-free cys67-mediated disulfide-bonded homodimers ($B27_2$) was first observed when refolding recombinant HLA-B27 in vitro(56). Several forms of B27 free heavy chains (FHC) including $B27_2$ are also expressed at the cell surface in cell lines following endosomal recycling of heterotrimers(70). The ability of B27 to form disulphide bonds through its unpaired cysteine at position 67 is both highly unusual for HLA- class 1 molecules and important for cell surface homodimer expression(70), although B27 as well as other HLA allotypes have been shown to be capable of homodimerization under appropriate conditions with roles for other cysteine residues demonstrated(58). Cell surface $B27_2$ and FHC bind to innate immune receptors on T, NK and myeloid cells. These include the Killer immunoglobulin receptors KIR3DL1, KIR3DL2 and LILIRB2 in humans (71) and the rodent Paired Immunoglobulin Receptors (PIR)(72). The binding specificity of $B27_2$ and B27 FHC for these receptors is different to that of heterotrimeric HLA-B27 complexes, which whilst binding KIR3DL1 and LILRB2 are also ligands for LILRB1 but do not bind KIR3DL2 with significant affinity. Furthermore $B27_2$ and FHC bind with higher affinity/avidity than other ligands to both LILRB2(73) and to KIR3DL2(74). The KIR3DL2/B27 interaction can have pro-inflammatory effects on both NK (75) and T cells(74), and is associated with a Th17 phenotype in AS(76). This model is shown in cartoon form in **Figure 3.**

Whilst these observations have led us to propose that cell surface $B27_2$ contributes to the pathogenesis of AS/SpA, there was until recently little direct evidence in human disease, in part due to the lack of reagents specific for

$B27_2$. Increased HLA class 1 heavy chain expression on peripheral blood monocytes of AS patients had been demonstrated using the HC10 antibody(77),(78). HC10 binds $B27_2$ and FHC but also recognizes other B27 heavy chain structures and binds to other HLA-B, C and some A allele heavy chains(79). Using a phage display library, novel antibodies with specificity for B27 heavy chains including $B27_2$ have been generated ((80) and Marroquin, Renner, Bowness unpublished data). One of these, HD6 binds to recombinant $B27_2$ complexes but not to HLA-B27 heterotrimers, HLA-A2, HLA-A3, HLA-A24 or -B7 complexes(80). HD6 also stains some but not all HLA-B27-transfected B lymphoblastoid cell lines, including the LBL721.220B27, LBL721.221B27 and C1RB27 lines. The binding pattern to recombinant and cell-expressed molecules is thus different to that seen with HC10. HD6 expression on monocytes from AS patients is significantly higher than that of monocytes from B27+ or B27- healthy control individuals(80). Lastly HD6 also inhibits binding of recombinant and cell-expressed $B27_2$ to immunoreceptors, raising the possibility of future therapeutic use(80). Nevertheless a number of outstanding questions remain unanswered regarding “homodimer” hypothesis of B27 pathogenesis. What are the cellular requirements for $B27_2$ formation? Does $B27_2$ formation in patients with Ankylosing Spondylitis correlate with disease activity and/or tissue specificity? Does blockade of B27 FHC interaction with immunoreceptors ameliorate disease in B27 transgenic animals? Addressing these questions will determine the validity of the B27 FHC hypothesis of SpA pathogenesis.

3.4 Other theories of HLA-B27 pathogenesis

3.4.1. Microbial dysbiosis and increased gut inflammation.

Studies from several groups have demonstrated subclinical gastrointestinal inflammation, both acute and chronic, in approximately half of AS patients(81). This is in addition to the well-recognized co-occurrence of AS and Crohn's disease and Ulcerative Colitis. Whilst the author favours the concept that other genetic (and environmental) factors predispose to GI inflammation in combination with AS, it remains a clear and testable possibility that the presence of HLA-B27 could itself alter the gut microbiome or local inflammatory response. It is noteworthy that from the available GWAS data (which are less comprehensive in AS than IBD) AS does not share autophagy-related genetic associations (e.g. ATG6) with Crohn's disease.

3.4.2. Thymic selection.

Alternatively HLA-B27 my act during T cell development in the thymus to facilitate generation of a pro-arthritic T cell repertoire (this is assumed in the "arthritogenic" peptide model). It is also possible that HLA-B27 may self present a portion of its own structure to the immune system(82), or may act as a receptor for a pathogen that triggers disease. Because of the tight linkage disequilibrium within the MHC region of chromosome 6, it has been suggested that B27 is a marker of a distinct but genetically linked pathogenic gene. However this possibility is now almost certainly excluded by the latest GWAS data (together with the transgenic rat data).

3.4.3 Amyloid generation. Lastly it has been proposed that HLA-B27 complexes might be predisposed to shed ß2m in certain anatomical sites which might then go on to form amyloid deposits(83).

3.5 Interaction of HLA-B27 with ERAP1 and other peptidases in AS pathogenesis

The genetic associations with AS of four aminopeptidases, including ERAP1(32), ERAP2 and LNPEP (both ER aminopeptidases) and the cytoplasmic peptidase NPEPPS (33), have refocused attention on the central role of HLA-B27 and peptide binding in disease pathogenesis. Of particular note the ERAP1 association, which is the second strongest after HLA-B27 and contributes approximately 15% of the population attributable risk, is only found in B27-positive AS patients(32). A similar epistatic interaction with ERAP1 has also been described in psoriasis for HLA Cw3(84), and for Behcet's disease and HLA-B*51(85), suggesting the possibility of similar HLA-driven pathogenic mechanisms operating in these diseases, even if precise ERAP1 allelic associations may differ in different conditions.

How do HLA-B27 and ERAP1 interact to cause AS? The only confirmed function of ERAP1 is to trim peptides for binding to HLA class 1 molecules. ERAP1 acts as a "molecular ruler" to trim the N terminus of peptides within the ER(86). ERAP1 allelic variants may thus play a key role in AS pathogenesis either directly through alteration of the repertoire of peptides bound to HLA-B27(87), or indirectly from the generation of abnormal

intracellular or extracellular forms of HLA-B27, due to altered (peptide-mediated) stability or trafficking. Altered MHC stability and immunogenicity have been demonstrated in ERAAP-deficient murine cells(88).

The crystal structure of ERAP1 has recently been solved including in both “open” and “closed” conformations -the latter with the protease inhibitor bestatin in the active site (89). ERAP1 has a non-redundant role in shaping the repertoire of peptides bound to HLA-B27(90), and the AS-protective K528R allele has reduced function in trimming extended peptides containing known HLA-B27 epitopes(89). ERAP1 polymorphisms may alter the repertoire of peptides loaded onto HLA-B27 and modulate subsequent immune recognition of bound peptides by cytotoxic T lymphocytes and/or natural killer cells. Alternatively ERAP1 polymorphisms might promote AS by affecting either endoplasmic reticulum misfolding or the export of pro-inflammatory B27 forms to the cell surface. Several questions arise which are under active investigation. Firstly it is not entirely clear if HLA-B27 misfolding increase in the presence of disease-associated ERAP1 variants (and reduce with ERAP1-protective variants). Secondly are more surface HLA-B27 heavy chains expressed in the presence of disease-associated ERAP1 variants, as suggested by Haroon and colleagues(91)? Finally it is not known if ERAP1 interacts directly with HLA-B27 and/or the peptide-loading complex and if this is altered for ERAP1 variants. None of these studies have yet been carried out in the context of ERAP2, LNPEP or the cytoplasmic peptidase NPEPPS, although all might be expected to have effects on the peptide repertoire available to bind HLA-B27.

Although incomplete these data provide strong support for a key role for antigen presentation and peptide generation in AS pathogenesis, conceptually placing HLA-B27 at the very centre. They also suggest that inhibition of ERAP1 might be a valid therapeutic strategy for treatment of AS and SpA. Indeed ERAP1 inhibitors are already in preclinical development[54].

Summary

AS is a largely inherited disease, almost certainly with a ubiquitous environmental trigger. Many genes contribute to AS pathogenesis, with HLA-B27 by far the most important. HLA-B27 may cause AS by presenting ”arthritogenic” peptides to cytotoxic T cells (or to NK cells through peptide-sensitive NK receptor recognition). B27 may “behave badly” to misfold within the ER and trigger stress or autophagy responses. Alternatively B27 may cause pathology through cell-surface expression of aberrant free heavy chain forms. The latter forms of HLA-B27 have been shown capable of driving pro-inflammatory Type 17 responses through interaction with KIR receptors expressed on T and NK cells.

SUMMARY POINTS

1. HLA-B27 is the strongest risk factor for development of Ankylosing Spondylitis; AS is a largely inherited disease.

2. HLA-B27 is a Human Leukocyte Antigen class 1 molecule which efficiently binds and presents immunodominant peptide epitopes to cytotoxic T cells in several important viral infections, including Influenza, HIV, EBV and hepatitis C.

3. HLA-B27 may cause AS by presenting ”arthritogenic” peptides to cytotoxic T cells.

4. B27 misfolds within the ER and is capable of triggering stress and autophagy responses. The former can result in IL-23 production.

5. Aberrant free heavy chain forms of HLA-B27 including homodimers are expressed at the cell-surface in cell lines and AS patient monocytes.

6. Free heavy chain forms of HLA-B27 bind KIR receptors expressed on T and NK cells and LILR receptors on myeloid cells.

7. Interaction of HLA-B27 free heavy chain forms with KIR3DL2-expressing T cells can drive type 17 immune responses.

FUTURE ISSUES

1. Are HLA-B27-restricted T cells with novel specificity present in the joints of SpA patients?

2. Does HLA-B27 misfolding increase in the presence of disease-associated ERAP1 variants?

3. Are more surface HLA-B27 heavy chains expressed in the presence of disease-associated ERAP1 variants

4. Does blockade of B27 FHC interaction with immunoreceptors ameliorate disease in B27 transgenic models of disease?

5. What if any are the effects of HLA-B27 on the gut microbiome or local inflammatory response?

6. What are the roles in disease of ERAP2, LNPEP and the cytoplasmic peptidase NPEPPS?

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Table 1 HLA B27-associated spondyloarthritides. The spondyloarthritides comprise a group of diseases sharing key clinical features and an HLA-B27 association.

Disease	Clinical features	HLA-B27 frequency % (approximate)	ref
Ankylosing spondylitis	Inflammation and new bone formation involving sacroiliac and spinal joints	94% (OR 171)	(39)
Reactive arthritis	Non-septic large joint arthritis following certain gastrointestinal or genitourinary bacterial infections	30-75%	(36)
Colitis-associated spondyloarthritis	Sacroiliac, spinal and large joint arthritis associated with Crohn's and Ulcerative colitis	33-75%	(93)
Psoriatic spondyloarthritis	Sacroiliac, spinal and large joint arthritis associated with skin psoriasis	40-50%	(93)
Juvenile enthesitis-related arthritis	Large joint arthritis associated with enthesitis usually presenting in teenage boys	76%	(94)
Acute anterior uveitis (AAU)	Acute sterile inflammation of the anterior chamber of the eye	50%	(38)

Table 2. HLA-B27 subtypes in different populations and their disease associations.

Country/Racial group	HLA-B27 subtype	Ref
Europe/US Caucasian	[a, b] **B*2705 B*2702**	(40)
China (Han)	**B*2705 B*2704**	(41)
India	B*2706 **B*2704 B*2705 B*2707**	(42),(95)
Thailand	B*2706 **B*2707 B*2704**	(42)
West Africa (Gambia)	**B*2703 B*2705**	(96)
Sardinia	**B*2705** B*2709 **B*2707 B*2702 B*2713**	(43)

[a]Most abundant subtype is shown first.
[b]Disease-associated subtypes are shown in bold.

Table 3. Theories explaining pathogenic role of HLA-B27 in AS

Theory	Supportive evidence	Evidence against
Arthritogenic peptide	Unique B27 peptide specificity. HLA-B27-restricted CD8 immune responses to bacteria known to trigger ReA,(48) . ERAP1 and (amino) peptidase disease associations(32),(33).	CD8 alpha depleted or -/- B27 TG rats still develop disease(53),(54).
ER stress	IL23 sufficient to cause enthesistis in mice(55) Chalmydia and other intracellular bacteria can trigger ER stress and IL23 production(63).	Excess beta 2m relieves colitis but not arthritis in B27 TG rats(66)
Cell surface free heavy chain (including) Homodimer recognition	Cell surface B27 FHC are expressed in SpA(71),(80). B27 FHC are strong ligands for KIR(74) and LILR(73). Increased NK and T lymphocytes KIR3DL2 expression in SpA (75).	No direct evidence of pathogenicity in humans or animal models.
Microbial dysbiosis (increased gut inflammation)	Evidence of local gut inflammation in >60% of AS patients	No evidence of B27 role or association. AS does not share autophagy-related genetic associations with Crohn's disease