Pathogenesis of ankylosing spondylitis and reactive arthritis

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Purpose of review

The hallmark of ankylosing spondylitis is acute and chronic spinal inflammation initiating in the sacroiliac joints, often coupled with enthesitis, presenting as chronic inflammation at the sites of ligamentous and tendinous insertions into bone. Peripheral joint synovitis can be a prominent feature as well. Reactive arthritis is a sterile synovitis arising after an extra-articular infection of enteric or urogenital tracts. HLA-B27 has been known for about the past 30 years to be associated with ankylosing spondylitis and reactive arthritis, but the pathogenesis of ankylosing spondylitis and reactive arthritis is still not well defined. Although the clinical manifestations of ankylosing spondylitis and reactive arthritis may differ, this update discusses the two diseases together and focuses on recent evidence in both.

Recent findings

With respect to HLA-B27 several recent studies address arthritogenic peptides, molecular mimicry, and aberrant forms of B27. Several candidate genes in addition to B27 have been implicated in recent genetic studies. With respect to bacterial infection, recent findings in bacterial antigenicity, host response through interactions of antigen-presenting cells, T cells, and cytokines are providing new understanding of host–pathogen interactions and the pathogenesis of arthritis. Endogenous host factors such as proteoglycans may play a role as autoantigens and contribute to chronic inflammation on that basis.

Summary

Recent advances provide additional new insights into distinct pathogenetic mechanisms in AS and ReA that arise from a complex interplay between genetic factors including HLA-B27 and environmental factors.

Keywords

bacterial infection, HLA-B27, T cells

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Abbreviations

AS	ankylosing spondylitis
HSP	heat shock protein 60
β_2m	β_2-microglobulin
MHC	major histocompatibility complex
SpA	spondyloarthropathy
TNF-α	tumor necrosis factor-α

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Introduction

The term *spondyloarthropathy* (SpA) was introduced to describe a family of arthritides sharing certain clinical features and having a strong association with HLA-B27. The recognition that HLA-B27 was associated with ankylosing spondylitis (AS) dates back 30 years, and many studies have focused on the biochemistry and immunology of HLA-B27, including genetic polymorphisms, infections, T-cell responses, and cytokines in the pathogenesis of SpA. The pathogenesis of SpA is as yet unexplained, however.

Spondyloarthropathy includes AS, reactive arthritis (ReA), psoriatic arthritis, arthritis related to inflammatory bowel diseases, as well as undifferentiated SpA. AS is a progressive disease in which chronic inflammation can lead to extensive new bone formation throughout the spine. In AS more than 90% of patients are HLA-B27 positive. ReA is thought to be triggered by urogenital and gastrointestinal infections and it shares with AS a strong association with HLA-B27. In this review, we provide a brief overview of pathogenesis of AS and ReA focusing on recent advances.

HLA-B27

HLA-B27 consists of a heavy chain having three α-domains, which noncovalently binds short peptides and β_2-microglobulin (β_2m) [1,2]. There are 24 HLA-B27 subtypes currently recognized. The structural patterns are consistent with B2705 being the ancestral allele and the other types being generated by small mutations [1]. B2705 is the dominant subtype and is associated with AS across broad ethnic and geographic boundaries. Of the subtypes studied to date, it appears that B2706 and B2709 do not confer susceptibility to AS. Although the HLA-B27 has remained a center of extensive research, the mechanism whereby HLA-B27 confers susceptibility to AS is not well defined. Current hypotheses regarding the pathogenesis of AS and ReA have sought to incorporate HLA B27 into mechanistic models.

Several different hypotheses have been proposed. In the arthritogenic peptide theory, HLA-B27 binds unique peptides of microbial or self-origin and presents them to $CD8^+$ T cells [1]. These peptides usually have an anchor arginine residue at their second position and the side chain of arginine is bound in the B pocket of HLA-B27 [2]. It was recently reported that $CD4^+$ T cells may be involved in class I–restricted immune recognition [3•]. Consequently AS could involve an HLA-B27-restricted $CD8^+$ T-cell or $CD4^+$ T-cell response to microbial or

self-peptides [4–6]. The principle of molecular mimicry is still proposed as a possible mechanism in B27-related pathogenesis. This postulates that the antibodies directed against foreign antigens arising during a bacterial infection are cross-reactive with HLA-B27. Recent reports have addressed molecular mimicry in AS. Fiorillo *et al.* [7•] recently showed allele-dependent similarity between a viral and a self-peptide derived from vasoactive intestinal peptide receptor (VIPR) 1 presented by HLA-B27 subtypes carrying the B*2709 or B*2705 alleles. The crystallographic study by Hulsmeyer *et al.* [8••] sheds light on the possible structural basis of differential susceptibility to AS conferred by different B27 subtypes. HLA-*B2705, an AS-associated subtype, has the capacity to bind a candidate autoantigen in two different conformations, in a way that is not shared by HLA-*B2709, which is a non-AS-associated subtype. Peptides from HLA-B27 have sequence homology with peptides from enterobacteria [9], *Chlamydia* [10], and cytokeratin [11]. It has also been reported that *Chlamydia* may reactivate autoreactive cytotoxic T lymphocytes with specificity for HLA-B27 [12].

There has been considerable interest in aberrant processing or folding of the heavy chain of HLA-B27. Under normal circumstances cell surface HLA-B27 consists of a heavy chain bound to β_2m and peptide. This complex is formed in the endoplasmic reticulum [13]. Heavy chain folding of HLA-B27 appears to be slower compared with other HLA alleles, however, possibly because of specific amino acid residues in the B pocket [14]. This misfolded heavy chain is usually removed in endoplasmic reticulum, but in the event of insufficient or unavailable chaperone, peptide, or β_2m, misfolded heavy chains are increased. This may increase expression of the protein BiP and generate an unfolded protein response in the endoplasmic reticulum, leading to activation of nuclear factor-κB [15]. In studies of transgenic rats, disulfide-linked intracellular heavy chain complexes are more prone to form and bind BiP in disease-prone wild-type B27 rats than in disease-resistant HLA-B7 rats [16••]. The data support the notion that accumulation of misfolded B27 may contribute to the pathogenesis of B27-associated disease. It should be noted that this slow folding may not be limited to HLA-B27 and viral infection can influence misfolding. The latter is not thought to contribute to the pathogenesis of AS or ReA, however, and further studies will be needed to resolve this issue [1]. In addition to misfolding within the endoplasmic reticulum assembly process, there is evidence that HLA-B27 is distinctive in its propensity to form heavy chain homodimers on the cell surface. Whether these homodimers could function as peptide-presenting structures has not been resolved, but these homodimers appear to be ligands for paired immunoglobulinlike receptors [17••].

Abnormal forms of HLA-B27 may react with $CD4^+$ T cells or natural killer cells, rather than $CD8^+$ T cells. The HLA-B27 molecule appropriately loaded with peptide usually interacts with $CD8^+$ T cells. HLA-B27-restricted $CD8^+$ T cells are unlikely to serve as effector cells in the transgenic rat model of HLA-B27-associated disease, however, because it was shown that $CD4^+$ T cells were capable of inducing arthritis [18]. Adoptive transfer studies showed $CD4^+$ T cells to be more important than $CD8^+$ T cells for arthritis [19]. Recently Roddis *et al.* [20••] has found that $CD4^+$ as well as $CD8^+$ T-cell responses are induced in B27-transgenic mice and HLA-B27-restricted alloreactive $CD4^+$ T cells were demonstrated with human cells and cell lines [3•,21]. HLA class I molecules are ligands for members of the killer immunoglobulin receptor and immunoglobulinlike transcript families (KIR3DL1, ILT4, and LIR6), which are expressed on natural killer cells, T cells, or monocytes [22]. As mentioned previously, HLA-B27, which exists on the cell surface as a classical heterodimer with β_2m, or as a less conventional heavy chain homodimer, acts as a ligand for these immune receptors although the affinities with HLA-B27 differ [17••,22,23], but evidence is lacking to define the pathogenetic significance of this interaction.

A recent hypothesis is that of autodisplay [24•]. In this construct, β_2m-free, peptide-free heavy chains support a helix-coil transition from α2 domain of HLA-B27 to α3 domain, facilitating rotation of backbone angles around residues 167 / 168, thereby occupying the molecule's own peptide binding cleft. This autodisplay of HLA-B27, occurring either within B27 molecules or between B27 molecules, might be the grounds for self-perpetuating inflammatory and immune stimulation.

Finally, there is a theory invoking β_2m deposition [25]. Cells bearing AS-associated HLA-B27 subtypes exhibit a higher rate of β_2m dissociation from surface HLA-B27 complex than non-AS-associated HLA-B27 subtypes. It is postulated that release of β_2m from a subpopulation of cell surface–expressed HLA-B27 molecules leads to β_2m deposition within the synovium and to the initiation of chronic inflammation on that basis.

The quest for the critical arthritogenic peptides continues to attract researchers in AS and ReA. Although there is evidence of HLA-B27-bound peptides, derived either from self or microbes, their arthritogenic capacity has proved difficult to define.

Genes other than HLA-B27

Several recent studies have been reported on genes other than HLA-B27 in AS [26•]. HLA-B60 was reported to be an important contributor to AS susceptibility and may act as an independent factor in B27-positive and B27-negative AS patients [27,28]. There was a report that HLA-DR1 is associated with AS [29]. Large multifunctional proteases and transporters associated with antigen presentation act

as chaperones for peptide transport and have been studied in the context of AS susceptibility, but with varying conclusions [30,31]. CARD15, which is thought to function as an intracytosolic toll-like receptor, has been associated with Crohn disease and psoriatic arthritis, but this appears not to be associated with AS [32,33]. Tumor necrosis factor-α (TNF-α), ank, matrix metalloproteinase-3, transforming growth factor-β, interleukin-1, and interleukin-1RN polymorphisms have been examined as possible candidate genes for susceptibility to AS, and there have been informative studies reporting both positive and negative results with these candidate genes [34–38,39•,40]. Evidence is compelling for genetic susceptibility to AS beyond HLA-B27 and the identity of these additional genes remains an active area of research. Well-controlled large studies are needed to pursue some of these promising leads.

Infection

Although ReA, another B27-related SpA, has a clear relation to antecedent infection, this is less clear for AS. B27-transgenic rats raised in a germ-free environment do not develop inflammatory pathology in the gut or the joints, and induction of arthritis following reintroduction of commensal gut flora supports the notion that such organisms play an important role in the pathogenesis of B27-associated gut and joint inflammation [41]. The following considers the role of infection with respect to bacteria and host immune response.

Bacteria

Evidence of previous infection can be defined in approximately 60% of ReA cases. The commonest triggering agents are *Chlamydia* organisms in the case of urogenital tract infections and gram-negative bacteria (e.g., *Shigella, Salmonella, Yersinia*, and C*ampylobacter)* in the case of gastrointestinal infections [42•] but important regional difference may occur [43]. *Chlamydia* is the commonest causative agent in ReA. *Chlamydia* DNA, mRNA, rRNA, and intact *Chlamydia*-like cells have been found in synovial tissues and peripheral blood [44–46]. The mechanisms accounting for persistence of *Chlamydia* infection, whereby avoidance of host immune clearance, have been proposed as follows [47,48]. In the chronic persistent state there is altered regulation of specific *Chlamydia* genes with reduced expression of major outer membrane protein and increased expression of heat shock protein 60 (HSP) and lipopolysaccharide. *Chlamydia* has the capacity to downregulate surface major histocompatibility complex (MHC) expression in infected cells [47]. Recent investigations have reported that interferon-γ reduces tryptophan, which plays an important role in chlamydial growth by enzyme reduction [47]. *Chlamydia* also inhibits of apoptosis of host cell by cytochrome c reduction and can induce T-cell apoptosis by local production of TNF-α [47,49,50]. Several factors likely affect the persistence of *Chlamydia* including direct stimulation of *Chlamydia*-upregulated proinflammatory soluble mediators. New techniques such as HLA-B27 tetramers with greater sensitivity have been applied to the detection of low-frequency antigen-specific T cells in *Chlamydia*-induced arthritis [51].

In enteric forms of ReA, the monocyte may serve as a reservoir or as a transporter of bacteria to synovial tissue [42•]. New analytical techniques have been applied to probing synovial fluids and tissue for evidence of prior or current microbes [52,53]. Invasion by *Salmonella,* however, did not alter the B27 peptide presentation profile [54]. Quantitative analysis of invasion of gram-negative bacteria into human synoviocytes did not correlate with the B27 status of the target cells, in contrast to prior studies using B27-transfected cells as targets [55]. Serologic studies have previously implicated certain gram-negative bacteria, notably *Klebsiella pneumoniae*, in the pathogenesis of AS. One recent analysis, which addressed both humoral and cellular host immune responses to candidate pathogens, found no evidence to support the notion that *K. pneumoniae* has a distinct pathogenic role in AS [56•].

Lipopolysaccharide in synovial tissue is a potent macrophage stimulator and can induce a range of inflammatory cytokines, largely via the nuclear factor-κB pathway [42•]. Lipopolysaccharide also induces monocyte chemotactic protein [57], enhances secretion of the neutrophil chemotactic and activating cytokine interleukin-8 from chondrocytes [58], and decreases C5aR expression on monocytes [59]. This sets the stage for persistence of activated macrophages within the synovium and the ensuing chronic inflammation. One unresolved issue is how an antecedent infection might induce inflammation and erosions in a joint such as the sacroiliac joint in the absence of viable organisms. Synovial fibroblast might play an intermediary role in this sequence of events, in light of the recent observation that synovial fibroblasts infected with *Salmonella typhimurium* mediate osteoclast differentiation and activation [60••].

Host factors

As described previously, the HLA-B27 is detected with five to 10 times greater frequency in ReA than in the general population [42•]. One important unresolved issue is whether HLA-B27 confers a selective host advantage or disadvantage in host responses to pathogens. In this regard it is of interest that HLA-B27 may confer a relative protective role against HIV infection [61]. Whether this experience in viral infections can be translated to bacterial infections is not yet resolved. It has been observed that there is enhanced intracellular replication of *Salmonella* in HLA-B27-expressing monocytic cells [62•]. HLA-B27 may also decrease the costimulatory function of antigen-presenting cells, as reported in the B27-transgenic rat model [63•]. This latter may lead to loss of tolerance toward microbial flora. *Salmonella* has been shown to promote expression

of the transcription factor activating protein 1 and to modulate signal transduction in HeLa cells with HLA-B27 [64]. Consequently, HLA-B27 affects the cell's ability to resist the bacteria and to mount a successful host defense.

Cytokines

Among host cytokines active in host defense against intracellular pathogens, three have received the most attention recently by investigators. TNF-α and interferon-γ are potent antibacterial Th1 cytokines, whereas interleukin-10 is a Th2 cytokine [65]. A significant reduction in the expression of Th1 cytokines was detected in HLA-B27 AS patients [66]. At onset of ReA, low production of TNF-α was observed in peripheral blood. Impaired Th1 cytokine production may delay elimination of bacteria, leading to persistence of the pathogen. Tumor necrosis factor receptor (TNFR) p55 knockout mice develop more severe arthritis after *Yersinia* infection [67]. Butrimiene *et al.* [68•] showed that in chronic ReA TNF-α production was higher and TNF-α-positive and interferon-γ-positive $CD3^+$ cells were significantly higher, suggesting that the Th1 response in chronic ReA is more dominant than that in acute ReA. In ReA, both $CD8^+$ and $CD4^+$ cells exhibit a Th1 profile with higher production of TNF-α and interferon-γ, suggesting that T cells contribute to pathogenesis by inducing a Th1 cytokine [69]. These studies need to be interpreted in the light of the fact that anti-TNF-α therapy may be an effective treatment of chronic ReA.

Other host factors

The extracellular matrix of articular cartilage is primarily composed of type II collagen and proteoglycan. Aggrecan is an important protein in fibrocartilaginous regions of tendons, which are target sites for inflammation in AS and ReA. Proteoglycans may prove to be important contributors to inflammation in AS. Aggrecan has three globular domains that bind chondroitin sulfate and keratan sulfate. Versican is an important proteoglycan and exhibits considerable homology with human aggrecan [70]. In a murine model of AS using cartilage proteoglycan immunization, Shi *et al.* [71] demonstrated that animals with peripheral and axial inflammation manifested immunity to aggrecan, whereas spinal inflammation and sacroiliitis without appendicular involvement were associated with immunity to versican. Kuon *et al.* [72•] analyzed $CD8^+$ T cells in response to aggrecan and identified new HLA-B27-restricted nonamer peptides. The structural aspects of this interaction have recently been studied and have observed that the residue Cys^{67} plays an important role in T-cell recognition of aggrecan peptides. These studies provide supportive evidence that HLA-B27-restricted epitopes derived from human aggrecan or versican may be involved in the induction of inflammation.

Baeten *et al.* [73••] showed that in SpA synovial tissues there are increased numbers of $CD163^+$ macrophages and local production of soluble $CD163^+$ when compared with rheumatoid arthritis patients, and these findings are associated with the degree of inflammation. This finding suggests that these $CD163^+$ cells play a functional role in early inflammation of AS and ReA.

Conclusion

HLA-B27 stands as the earliest and most robust genetic marker associated with a rheumatic disease. Although the association with AS dates back three decades and there have been marked advances in the research tools of immunology and biochemistry, the pathogenesis of AS and ReA and role of HLA-B27 have not been definitively solved. The pursuit of this goal has implications not only for the SpA but also for resolving pathogenic mechanisms for new genetic markers of susceptibility in arthritis in general.

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