A review of the pathogenesis of ankylosing spondylitis

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✓ Ankylosing spondylitis (AS) is a chronic, progressive inflammatory rheumatic disease involving primarily the sacroiliac joints and the axial skeleton. The main clinical features are back pain and progressive stiffness of the spine. Oligoarthritis of the hips and shoulders, enthesopathy, and anterior uveitis are common, and involvement of the heart and lungs is rare. The current understanding of the pathogenesis of this disorder is limited. Despite the strong association between human leukocyte antigen B27 (HLA-B27) and susceptibility to AS reported over the past 30 years, the exact pathogenic role of HLA-B27 in AS and other spondyloarthropathies has yet to be determined. The authors present a review of the literature pertaining to the pathogenesis of AS over the past several decades.

Ankylosing spondylitis is a polygenic disorder, with HLA-B27 playing a critical causative role in its pathogenesis. Animal studies of the immunobiology of HLA-B27 have provided significant insight into the pathogenic role of HLA-B27. The search for the antigenic peptide to support the “arthritogenic peptide” hypothesis has been disappointing. Over the past decade there has been increasing interest in the critical role of the misfolding and unfolded protein response of the heavy chain HLA-B27 in the modulation of the inflammatory response. Although there have been significant new findings in the understanding of the pathogenesis of AS, the exact mechanisms have yet to be identified. There is considerable optimism that additional susceptibility genes, predisposing factors, and regulators of the inflammatory process will be identified that will provide avenues for future treatment. (DOI: 10.3171/FOC/2008/24/1/E2)

KEY WORDS • ankylosing spondylitis • HLA-B27 • pathogenesis • unfolded protein response

ANKYLOSING spondylitis is a major subtype of a group of chronic inflammatory diseases known as spondyloarthropathies. It affects young adults with the peak age of onset between 20 and 30 years. Men are more often affected than women, with a ratio of approximately 3:1.⁸ About 80% of patients with AS develop the first symptoms before their third decade of life and < 5% present in the fourth decade of life. Patients with juvenile-onset AS become symptomatic at or before 16 years of age. The prevalence of AS is 0.1–1.4%, and this is dependent on the ethnicity, the prevalence of HLA-B27, the selection of patients for evaluation, and the screening criteria used for diagnosis.¹⁶ This condition is more common in persons of northern European heritage and least common in sub-Saharan Africans. Although it is important to recognize the strong correlation between the prevalence of HLA-B27 and AS in any given population, one must not forget that most individuals who test positive for HLA-B27 are healthy. Non-HLA-B27 genetic and environmental factors have an important role in the development and progression of this disease.

Clinical Features and Diagnosis

The primary clinical features of AS include inflammatory back pain caused by sacroiliitis, inflammation at other locations in the axial skeleton, peripheral arthritis, enthesitis, and anterior uveitis.^{19,21} Structural changes are caused mainly by osteoproliferation rather than osteodestruction. Syndesmophytes and ankylosis are the most characteristic features of this disease. The characteristic symptoms of AS are low-back pain, buttock pain, limited spinal mobility, hip pain, shoulder pain, peripheral arthritis, and enthesitis. Neurological symptoms can occur with cord or spinal nerve compression resulting from several complications of this spinal disease. Vertebral fractures can develop in patients with ankylosed spines with minimal or no traumatic injury. The most common fracture site is at the C5–6 interspace.³¹ Clinically significant atlantoaxial subluxation can occur in up to 21% of patients with AS and can lead to spinal cord compression.²³ Cauda equina syndrome is a rare complication of longstanding AS; its pathogenesis is poorly understood and includes inflammation, arachnoiditis, mechanical stretching, compression of the nerve roots, demyelination, and ischemia.²⁵

The diagnosis of AS is made from a combination of clinical features and evidence of sacroiliitis by some imaging technique defined by the 1984 Modified New York Criteria.

Abbreviations used in this paper: AS = ankylosing spondylitis; β 2m = β -2-microglobulin; ER = endoplasmic reticulum; HLA = human leukocyte antigen; MHC = major histocompatibility complex.

FIG. 1. Radiographs showing examples of Grade II (A), Grade III (B), and Grade IV sacroiliitis (C).

The clinical criteria are: 1) low-back pain and stiffness of > 3 months' duration that improves with exercise but is not relieved by rest; 2) limitation of motion of the lumbar spine in both the sagittal and frontal (coronal) planes; and 3) limitation of chest expansion relative to normal values corrected for age and sex. The radiological criteria are sacroiliitis Grade 2 or higher bilaterally, or Grade 3 or higher unilaterally. The radiographic grading of sacroiliitis consists of 5 grades (Fig. 1): Grade 0 is a normal spine; Grade 1 indicates suspicious changes; Grade 2 indicates sclerosis with some erosion; Grade 3 indicates severe erosions, pseudodilatation of the joint space, and partial ankylosis; and Grade 4 denotes complete ankylosis. Definite AS is present when 1 radiological criterion is associated with at least 1 clinical criterion. Probable AS is considered if there are 3 clinical criteria present or radiologic criteria exist with no signs or symptoms to satisfy the clinical criteria.³⁰

Genetic Factors

The exact cause of AS and other spondyloarthropathies is unknown, but genetic and environmental factors play an important role in their pathogenesis. The amount of genetic contribution to the risk of these disorders is variable. Two sizable twin studies of AS suggest a large genetic contribution to the risk of disease. The aggregate concordance of AS is 63% and 13% for monozygotic and dizygotic twins, respectively. From these concurrence rates it is estimated that approximately 90% of the risk of developing the disease is related to genetic makeup.^{4,13} A strong link between AS and the *HLA-B27* gene exists and has been definitively confirmed. Despite the fact that 90–95% of patients with AS are positive for HLA-B27, it accounts for less than one third of the genetic risk for AS.³ More than 90% of patients with AS are positive for 1 of the alleles of *HLA-B27*, while the risk of developing this disease is only about 5% in HLA-B27+ individuals without seropositive relatives. This risk is increased 5- to 16-fold if there is a first degree relative with AS.² Human leukocyte antigen B60 has also been implicated in the risk for AS in patients both positive and negative for HLA-B27 from Europe and Taiwan. Other MHC genes implicated in AS include *MICA*, *HLA-DRB1*/MHC Class II alleles, *tumor necrosis factor- α* , *IL-1ra*, and *low molecular weight proteasome (LMP)*.

Role of HLA-B27

Human leukocyte antigen B27 is an *HLA-B* allele of the MHC Class I molecules and is the most established genetic susceptibility marker for AS. The genes of the HLA locus are located on the short arm of chromosome 6. The *HLA-B27* gene designates a family of at least 31 closely related alleles, known as subtypes.^{24,26} Not all the subtypes are associated with AS; HLA-B*2705 is found in all populations, as is the parent HLA-B27 molecule. Most of the subtypes are a result of one or more amino acid substitutions mostly resulting from changes in exons 2 and 3 which encode the alpha-1 and alpha-2 domains of the heavy chain and along specific geographic patterns. The most common subtypes (HLA-B*2705, B*2702, B*2704, and B*2707) are associated with AS. The subtypes HLA-B*2706 and B*2709, which are found in Southeast Asia and Sardinia, respectively, are not associated with AS.

The major function of the HLA Class I molecules is to present antigenic peptides to the $\alpha\beta$ T-cell receptors on cytotoxic (CD8+) T lymphocytes. The HLA Class I molecule is composed of a 45-kD polymorphic heavy chain, noncovalently complexed with a soluble nonpolymorphic light chain, 12-kD monomorphic unit, β_2m . The heavy chain itself is composed of 3 domains, α_1 , α_2 , α_3 . The first 2 domains together form 2 antiparallel helices resting on a platform of an 8-stranded pleated sheet, which itself rests on 2 barrel-shaped structures derived from the third domain and β_2m . Resting inside the platform is an antigenic peptide that is usually 8–11 amino acids in length. These peptides are derived from endogenous proteins and from proteins of viruses and bacteria that have invaded the cells. The antigenic peptide is in contact with the heavy chain at several locations known as “pockets.” These pockets are designated A–F along the length of the platform. The feature that distinguishes *HLA-B27* from most other HLA Class I alleles is in the residues of the so-called B pocket of the heavy chain. This B pocket accommodates the second residue of the antigenic peptide. The glutamic acid residue lining this HLA-B27 B pocket is particularly important, dictating that the B pocket of HLA-B27 can accommodate only an arginine residue from the peptide. As a result, the most suitable peptide residue is arginine. Indeed, sequencing of HLA-B27 endogenous peptides shows that most antigenic peptides associated with HLA-B27 have arginine as the second residue.^{2,17,18,32}

FIG. 2. Flow chart showing the sequence of events in antigen presentation. Self- or antigen-proteins in the cytoplasm are degraded by proteasomes, and the short peptides are transported into the ER where they meet MHC Class I molecules. The folded MHC Class I molecule with its peptide is then transported to the cell surface via the Golgi apparatus. Recognition of the MHC-peptide complex by the T-cell receptors of an antigen-specific T lymphocyte completes the antigen presentation.

In an antigen-presenting cell, the MHC molecule presents the peptide derived from the antigen to the CD8+ T cell. The peptides are formed from the degradation of proteins in the cytoplasm by proteasomes. These short peptides are transported into the ER where they meet MHC Class I molecules. The folded MHC Class I molecule with its peptide is then transported to the cell surface via the Golgi apparatus. Recognition of the MHC-peptide complex by the T-cell receptor of an antigen-specific T lymphocyte completes the antigen presentation (Fig. 2).

Animal Models

Two animal models of HLA-B27-related arthritis have verified the central role of HLA-B27 in arthritis and suggested possible mechanisms of pathogenesis. Arthritis develops in transgenic mice that express human *HLA-B27* and $\beta 2m$, but not in those expressing human *HLA-B27* and murine $\beta 2m$.^{14,15} Another model demonstrates that the introduction of the *HLA-B27* gene plus the human $\beta 2m$ gene into rats results in a syndrome with features similar to those of human reactive arthritis, including inflammation of the peripheral and vertebral joints, male genital tract, gastrointestinal tract, skin, nails, and heart.¹⁰ In addition, the degree of susceptibility to inflammatory disease in an individual syngeneic rat line correlated with the level of *HLA-B27* gene expression in that line.²⁷ These models support the idea that *HLA-B27* is the genetic element that predisposes to arthritis and that the presence of *HLA-B27* alone is not sufficient for the development of animal spondyloarthritis. Environmental factors also have a role since transgenic rodents do not develop arthritis if they are raised in a germ-free or a pathogen-free environment. On the other hand, these arthritis-free animals develop inflammation when switched to a regular environment.²⁸

Arthritogenic Peptide Hypothesis

Human leukocyte antigen B27-specific CD8+ T lymphocytes have been the focus of many studies. Sharing of

FIG. 3. Flow chart demonstrating unfolded protein response. Heavy chains, $\beta 2m$, and peptides assemble in the ER to form an HLA molecule prior to being exported to the cell surface. Heavy chains that do not fold properly or misfold are disposed of by ER-associated degradation (ERAD). When unfolded proteins overaccumulate in the ER, an unfolded protein response is signaled.

T-cell receptors among different patients with spondyloarthropathies has been noted.²⁰ This finding suggests that these different patients are responding to the same antigens. The major hypothesis is that there are certain immunodominant arthritis-causing HLA-B27-specific antigenic peptides which are shared among the arthritis-causing pathogens, and that these peptides are also cross-reactive with autoantigens. Hence, when an HLA-B27+ individual is infected with an arthritis-causing pathogen, an HLA-B27-specific, cytotoxic T-cell-mediated autoimmune response would be initiated in the joints. This is known as the “arthritogenic peptide” hypothesis.

As early as 1993, CD8+ T lymphocytes were identified in the joints of patients with reactive arthritis that could recognize both bacterially infected and uninfected target cells.^{11,12} These findings prompted efforts to identify and sequence the bacterial peptides responsible for the CTL reactivity. For *Yersinia* and *Chlamydia* several bacterial peptides have been identified, but it remains to be determined whether any of these are cross-reactive with self-peptides, as postulated in the “arthritogenic peptide” hypothesis.

Another self-antigen that has been investigated is derived from cartilage. At least 18 cartilage proteins have been screened for potential reactivity, first by peptide-predicting computer programs, and then by actual testing with synovial T lymphocytes from patients with AS. A peptide derived from Type VI collagen was able to stimulate T lymphocytes obtained from 4 of 7 synovial fluid samples.¹ Other possible self and foreign antigenic peptides are those derived from human vasoactive intestinal peptide receptor-1 and the Epstein-Barr virus protein.⁶ These are the only pairs of self- and pathogen-derived HLA-B27 peptides that have been compared using x-ray crystallography.⁷ There is no conclusive evidence to prove that any of these peptides is the “arthritis-causing” peptide.

TABLE 1

Summary of pathological changes occurring in the joint that eventually cause AS

Anatomical Structure	Pathological Changes
synovium	synovitis pannus formation loss of synovium
bone marrow	myxoid changes granulation tissue formation replacement with eventual trabecular bone
cartilage	granulation tissue lysis, new cartilage formation endochondral bone replacement by trabecular bone
bone	lysis replacement by mature trabecular bone sclerosis
entheses	enthesitis new bone formation replacement by mature trabecular bone

The HLA-B27 Folding Hypothesis

Another possible explanation for the observation that HLA-B27 heavy chains could promote arthritis has been suggested. The proposed mechanism involves a proinflammatory response to overloading of the ER with misfolded proteins. HLA molecules mature inside a compartment of the cell designated as the ER. Assembly of a stable HLA molecule (heavy chain β 2m-peptide complex) in the ER is necessary before being exported to the cell surface. Following synthesis and glycosylation, free heavy chains are initially stabilized by chaperones (calreticulin and tapasin) until a conformation suitable to bind β 2m and a peptide is achieved. In the absence of β 2m, heavy chains ultimately misfold and are degraded. Endoplasmic reticulum-associated degradation is a quality control process of retrotranslocation of misfolded proteins from the endoplasmic reticulum to the cytosol, where there is deglycosylation and proteasomal degradation (Fig. 3.). Misfolding is described as an abnormal conformation of the heavy chain, yet proteins that are stalled in the ER because they have not yet folded properly are also considered misfolded.²⁶

The rate of folding is one of the determinants in the fate of the molecules. Human leukocyte antigen B27 is different from other HLA alleles in that it has a significantly slower rate of folding than many other proteins. In addition to its role in peptide selection, the B pocket has a dramatic effect on folding efficiency and can cause misfolding by directly influencing heavy chain folding or by altering its affinity for β 2m peptide complex. When unfolded proteins overaccumulate in the ER, an unfolded protein response is signaled and leads to generation of proinflammatory arthritis-causing cytokines and chemokines.^{5,22} The unfolded protein response begins with activation of nuclear factor- κ B which translocates to the nucleus and stimulates transcription of genes encoding cytokines such as tumor necrosis factor- α , interleukins (IL-1 and IL-6), and chemokines. This then leads to an inflammatory reaction in the joints.⁵ Indeed, an ER unfolded protein response has been demonstrated in macrophages derived from arthritic HLA-B27 transgenic rats.²⁹ In patients with spondyloarthritis, there is some evidence of an unfolded protein response in synovial fluid mononuclear cells.⁹

Conclusions

Our understanding of the pathogenesis of AS is limited. Ongoing investigation of the mechanisms of this disease process are focused on identifying initiating factors, downstream events, mediators of inflammation, and regulators of the process. It has been estimated that approximately 90% of the risk of developing AS is heritable. The most powerful of the genetic risk factors is related to the HLA-B27 molecule. Given the important role that HLA-B27 plays in risk, several possible mechanisms have been proposed. However, despite the intense interest and active investigation, there is yet no general consensus on how HLA-B27 contributes to disease susceptibility. The role of environmental factors remains elusive, as does the understanding of the propensity of AS to involve attachment of ligaments and tendons to bone (entheses) or the involvement of the sacroiliac joints.

References

- Atagunduz P, Appel H, Kuon W, Wu P, Thiel A, Kloetzel PM, et al: HLA-B27-restricted CD8+ T cell response to cartilage-derived self peptides in ankylosing spondylitis. **Arthritis Rheum** **52**: 892–901, 2005
- Baron M, Zendel I: HLA-B27 testing in ankylosing spondylitis: an analysis of the pretesting assumptions. **J Rheumatol** **16**:631–636, 1989
- Brewerton DA, Hart FD, Nicholls A, Caffrey M, James DC, Sturrock RD: Ankylosing spondylitis and HL-A 27. **Lancet** **1**: 904–907, 1973
- Brown MA, Kennedy LG, MacGregor AJ, Darke C, Duncan E, Shatford JL, et al: Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. **Arthritis Rheum** **40**:1823–1828, 1997
- Colbert RA: HLA-B27 misfolding: a solution to the spondyloarthritis conundrum? **Mol Med Today** **6**:224–230, 2000
- Fiorillo MT, Maragno M, Butler R, Dupuis ML, Sorrentino R: CD8(+) T-cell autoreactivity to an HLA-B27-restricted self-epitope correlates with ankylosing spondylitis. **J Clin Invest** **106**:47–53, 2000
- Fiorillo MT, Ruckert C, Hulsmeier M, Sorrentino R, Saenger W, Ziegler A, et al: Allele-dependent similarity between viral and self-peptide presentation by HLA-B27 subtypes. **J Biol Chem** **280**:2962–2971, 2005
- Gran JT, Husby G, Hordvik M: Prevalence of ankylosing spondylitis in males and females in a young middle-aged population of Tromsø, northern Norway. **Ann Rheum Dis** **44**:359–367, 1985
- Gu J, Rihl M, Märker-Hermann E, Baeten D, Kuipers JG, Song YW, et al: Clues to pathogenesis of spondyloarthritis derived from synovial fluid mononuclear cell gene expression profiles. **J Rheumatol** **29**:2159–2164, 2002
- Hammer RE, Maika SD, Richardson JA, Tang JP, Taurog JD: Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta 2m: an animal model of HLA-B27-associated human disorders. **Cell** **63**:1099–1112, 1990
- Hermann E, Yu DT, Meyer zum Büschenfelde KH, Fleischer B: HLA-B27-restricted CD8 T cells derived from synovial fluids of patients with reactive arthritis and ankylosing spondylitis. **Lancet** **342**:646–650, 1993
- Huang F, Hermann E, Wang J, Cheng XK, Tsai WC, Wen J, et al: A patient-derived cytotoxic T-lymphocyte clone and two peptide-dependent monoclonal antibodies recognize HLA-B27-peptide complexes with low stringency for peptide sequences. **Infect Immun** **64**:120–127, 1996
- Järvinen P: Occurrence of ankylosing spondylitis in a nationwide series of twins. **Arthritis Rheum** **38**:381–383, 1995

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14. Khare SD, Hansen J, Luthra HS, David CS: HLA-B27 heavy chains contribute to spontaneous inflammatory disease in B27/human beta2-microglobulin (beta2m) double transgenic mice with disrupted mouse beta2m. **J Clin Invest** 98:2746–2755, 1996
15. Khare SD, Luthra HS, David CS: Animal models of human leukocyte antigen B27-linked arthritides. **Rheum Dis Clin North Am** 24:883–894, 1998
16. Lawrence RC, Helmick CG, Arnett FC, Deyo RA, Felson DT, Giannini EH, et al: Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. **Arthritis Rheum** 41:778–799, 1998
17. Madden DR, Gorga JC, Strominger JL, Wiley DC: The structure of HLA-B27 reveals nonamer self-peptides bound in an extended conformation. **Nature** 353:321–325, 1991
18. Madden DR, Gorga JC, Strominger JL, Wiley DC: The three-dimensional structure of HLA-B27 at 2.1 Å resolution suggests a general mechanism for tight peptide binding to MHC. **Cell** 70:1035–1048, 1992
19. Martin TM, Smith JR, Rosenbaum JT: Anterior uveitis: current concepts of pathogenesis and interactions with the spondyloarthropathies. **Curr Opin Rheumatol** 14:337–341, 2002
20. May E, Dulphy N, Frauendorf E, Duchmann R, Bowness P, Lopez de Castro JA, et al: Conserved TCR beta chain usage in reactive arthritis; evidence for selection by a putative HLA-B27-associated autoantigen. **Tissue Antigens** 60:299–308, 2002
21. McGonagle D, Gibbon W, Emery P: Classification of inflammatory arthritis by enthesitis. **Lancet** 352:1137–1140, 1998
22. Mear JP, Schreiber KL, Munz C, Zhu X, Stevanovic S, Ramensee HG, et al: Misfolding of HLA-B27 as a result of its B pocket suggests a novel mechanism for its role in susceptibility to spondyloarthropathies. **J Immunol** 163:6665–6670, 1999
23. Ramos-Remus C, Gomez-Vargas A, Guzman-Guzman JL, Jimenez-Gil F, Gamez-Nava JI, Gonzalez-Lopez L, et al: Frequency of atlantoaxial subluxation and neurologic involvement in patients with ankylosing spondylitis. **J Rheumatol** 22:2120–2125, 1995
24. Reveille JD: The genetic basis of ankylosing spondylitis. **Curr Opin Rheumatol** 18:332–341, 2006
25. Sant SM, O'Connell D: Cauda equina syndrome in ankylosing spondylitis: a case report and review of the literature. **Clin Rheumatol** 14:224–226, 1995
26. Smith JA, Märker-Hermann E, Colbert RA: Pathogenesis of ankylosing spondylitis: current concepts. **Best Pract Res Clin Rheumatol** 20:571–591, 2006
27. Taurog JD, Maika SD, Simmons WA, Breban M, Hammer RE: Susceptibility to inflammatory disease in HLA-B27 transgenic rat lines correlates with the level of B27 expression. **J Immunol** 150:4168–4178, 1993
28. Taurog JD, Richardson JA, Croft JT, Simmons WA, Zhou M, Fernández-Sueiro JL, et al: The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. **J Exp Med** 180:2359–2364, 1994
29. Turner MJ, Sowders DP, DeLay ML, Mohapatra R, Bai S, Smith JA, et al: HLA-B27 misfolding in transgenic rats is associated with activation of the unfolded protein response. **J Immunol** 175:2438–2448, 2005
30. van der Linden S, Valkenburg HA, Cats A: Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. **Arthritis Rheum** 27:361–368, 1984
31. Vosse D, Feldtkeller E, Erlendsson J, Geusens P, van der Linden S: Clinical vertebral fractures in patients with ankylosing spondylitis. **J Rheumatol** 31:1981–1985, 2004
32. Yamaguchi A, Tsuchiya N, Mitsui H, Shiota M, Ogawa A, Tokunaga K, et al: 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** 38:1672–1677, 1995

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