The pathogenesis of ankylosing spondylitis

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✓ Ankylosing spondylitis (AS) is a chronic inflammatory disease that can cause significant functional complications by affecting the sacroiliac joints and axial skeleton. Despite a longstanding knowledge about the familial associations of this disease, particularly among patients positive for human leukocyte antigen (HLA)-B27, the fundamental pathogenetic mechanism by which this disease arises in genetically susceptible individuals remains ill defined. Furthermore, the molecular predilection for characteristic articular site involvement remains under ongoing investigation. Current theories about the HLA-B27 association range from the presentation of novel arthritogenic peptides, to abnormal autoimmune stimulation, to anomalous microbial tolerance. The immune effectors of this damage include CD4+, CD8+, and natural killer cells, with marked heterogeneity at different sites. Biomechanical stresses may trigger this disease by exposing the body to previously immune-sequestered autoantigens or by providing a route for bacterial seeding. Environmental triggers such as infection have not been definitively established but may represent a primary pathogenic step in a molecular-mimicry process. In this article, the authors review the current literature on the origin and pathophysiology of AS, focusing on genetic and molecular associations, consequent pathomechanisms, and associated triggers. An improved understanding of the sequence of molecular events that predispose and initiate the onset of this disease will allow for more specific and targeted therapy and better avoidance of the significant side effects of systemic immunomodulation. (DOI: 10.3171/FOC/2008/24/1/E3)

KEY WORDS • ankylosing spondylitis • pathogenesis • spondyloarthropathies • pathophysiology

ANKYLOSING spondylitis is a chronic inflammatory disease that primarily affects the sacroiliac joints and the axial skeleton. It is generally considered to be the archetypal seronegative spondyloarthropathy. While there is considerable ethnic and geographic variation in the prevalence of AS, the strong familial associations of this disease, particularly among patients positive for HLA-B27, have implicated a role for an immunogenetic abnormality in the pathogenesis. Despite significant advances in the diagnosis and management of AS, there continues to be much uncertainty concerning the specific genetic predispositions and environmental triggers that result in this disease. An improved understanding of the sequence of molecular events that predispose and initiate the onset of this disease will allow more specific and targeted therapy with avoidance of the significant side effects of systemic im-

munomodulation. We review the current literature on the pathophysiology of AS, focusing on genetic and molecular associations, consequent pathomechanisms, and associated environmental triggers. The pathological features of AS are also reviewed.

Genetic Associations and Molecular Pathogenetic Mechanisms

The predominant genetic association with AS is the MHC group of molecules, specifically HLA-B27. There are significant associations of AS with HLA-B27, and the estimated overall contribution to AS susceptibility is estimated to be between 20 and 40%. We will review the HLA-B27, other MHC, and non-MHC genetic associations with AS, and examine the pathogenetic mechanisms and the cytokine and enzyme effectors of inflammation. An understanding of these genes and their protein products (Table 1) provides insight into the underlying disease pathogenesis and may permit the development of novel avenues of treatment.

Human Leukocyte Antigen–B27

Human leukocyte antigen–B27 is an MHC Class I mol-

Abbreviations used in this paper: AAU = acute anterior uveitis; AS = ankylosing spondylitis; ER = endoplasmic reticulum; ERAD = endoplasmic reticulum–associated degradation; HLA = human leukocyte antigen; HSP = heat shock protein; IFN = interferon; IL = interleukin; MHC = major histocompatibility complex; MMP = matrix metalloproteinase; NK = natural killer; TGF = transforming growth factor; TNF = tumor necrosis factor; UPR = unfolded protein response.

TABLE 1

Genetic associations with ankylosing spondylitis

Gene Group	Products
HLA-B27	HLA-B27
non-HLA-B27 MHC genes	HLA-B60
	HLA-B61
	HLA-DRB1*01, B1*04
	HLA-DR8
	LMP-2, LMP-7
non-MHC genes	HSP-70
	IL-1 gene cluster
	CYP2D6
	TGFβ

ecule that is encoded on chromosome 6; although it is ubiquitous among cell types, its expression is higher on antigen-presenting cells. After translation and tertiary folding, this protein binds to β_2 -microglobulin and is loaded with an oligopeptide. These peptides are normally derived from self-proteins, but antigenic peptides may be displayed when intracellular microbes infect the cell. The trimolecular complex travels through the Golgi apparatus to the cell surface where the antigenic peptide is presented to CD8+ lymphocytes or NK cells. Human leukocyte antigen-B27 also has associations with other spondyloarthropathies, including reactive arthritis, psoriatic arthritis, and anterior uveitis. There is a strong genetic association between HLA-B27 and AS, with the protein isoform found in more than 90% of afflicted patients;^{19,92,99} however, fewer than 5% of HLA-B27+ individuals will develop AS.^{14,111}

The fact that only a small proportion of people positive for *HLA-B27* develop AS may be attributable to the different allele types that exist. There are at least 25 allele subtypes of *HLA-B27* that encode 23 different gene products. The most common subtype (B*2705) is thought to be the parent molecule from which the other types have evolved and is the most closely associated with the risk of AS.⁶ Other subtypes that confer disease susceptibility include B*2701, B*2702, B*2704, and B*2707, whereas the B*2706 and B*2709 alleles that are common in Southeast Asia and Sardinia do not have an association with AS.⁶

Another explanation for disease susceptibility in certain HLA-B27+ individuals may be the expression levels in antigen-presenting cells. Human leukocyte antigen-B27 has been found to be more highly expressed in peripheral blood mononuclear cells in HLA-B27+ patients with AS than in healthy HLA-B27+ individuals.²⁷ Furthermore, expression levels were found to be higher in AS patients with B*2705 than in healthy individuals with B*2709 and B*2705.

The Role of HLA-B27 in AS

Several theories have been promoted with regard to the molecular pathogenetic role of HLA-B27 in AS. These include the presentation of arthritogenic peptides, aberrant folding of surface heavy chains, HLA-B27 misfolding, and enhanced intracellular microbial survival.⁹¹ These mechanisms are summarized in Table 2 and explained in the following sections.

Presentation of Arthritogenic Peptides. Human leukocyte antigen-B27 may have the ability to bind unique antigenic

TABLE 2

Role of HLA-B27 in pathogenesis of ankylosing spondylitis

Hypothesis	Pathogenic Site	Immunity
arthritogenic peptide presentation	immunological	adaptive
aberrant surface heavy chain	immunological	innate
abnormal protein folding	intracellular	innate
enhanced microbial survival	intracellular	innate

peptides,^{38,40,62–65} leading to the CD8+ cytotoxic T-cell responses to these self or bacterial sequences. The ensuing cytolytic response leads to tissue injury and diffuse inflammation. Unlike most other MHC Class I molecules, there is a restriction for peptides with arginine at the P2 position to bind to HLA-B27.^{55,74,75} Single amino acid changes from aspartate in the B*2705 allele to histidine in the B*2709 allele leads to a loss of the association with AS.³¹ Thus, the correlation with AS may result from the B*2705 allotype's differential peptide-binding repertoire.⁸⁹ In general, subtypes not associated with AS have a restriction for nonpolar c-terminal residues such as aliphatic chains and phenylalanine, whereas disease-associated molecular subtypes are able to bind peptides with a c-terminal tyrosine.⁷²

The identity of the specific arthritogenic peptide in AS remains elusive. In support of molecular mimicry eliciting an untoward inflammatory reaction, Scofield and coworkers¹⁰⁰ have identified a nonapeptide of sequence LRRY LENGK that occurs both in HLA-B27 heavy chains and enteric bacteria. This nonapeptide has been demonstrated to be recognized by the peripheral blood lymphocytes of patients with AS who are positive for *HLA-B27*, but not by healthy individuals positive for *HLA-B27*.⁴⁴ Authors of molecular modeling studies have proposed that free HLA-B27 heavy chains can undergo backbone rotation to permit residues 169–181 to occupy its own binding groove.⁷³ Presentation of this epitope, either within or between HLA-B27 molecules, could elicit an autoimmune reactivity. Another self-peptide that has received attention is a 9 amino acid fragment of VIPR (residues 400–408), a sequence highly homologous to an Epstein-Barr virus-derived epitope of latent membrane protein 2 (residues 236–244). Although the CD8+ T-cell response to VIPR was greater in AS patients than in controls,³⁹ both the HLA-B27 subtypes with (B*2705) and without (B*2709) the AS association could present this molecule. However, crystallographic evidence suggests that while B*2709 can bind the fragment in the conventional binding groove, B*2705 can additionally present the peptide in a second conformation creating a salt-bridge with the differing aspartate-116 residue.⁴⁰ In addition, the LMP2 protein can also be uniquely presented in a nonconventional conformation by B*2705.

Proteomic analysis has also been used to evaluate human serum protein binding to HLA-B27 in 7 patients with AS and 7 healthy controls from the same Chinese family.⁷⁰ Four isoforms of prehaptoglobin were found to be highly expressed in all the study patients with active disease. The decapeptide VRYQCKNYYK was predicted to have a much higher binding affinity to HLA-B27 than conventional epitopes, and so it was concluded that prehaptoglobin is involved in the pathogenesis of AS. However, this

study was limited in that only the serum proteome was evaluated, while the high specificity of AS for specific tissues suggests that the differential protein expression in those tissues may demand investigation. Although these observations do not conclusively implicate any of these potential antigens, they do suggest a molecular mechanism by which disease-associated HLA-B27 subtypes may become pathogenic.

Another antigen of specific interest is aggrecan, a large aggregating chondroitin sulfate proteoglycan. A strong cellular immune response against the G1 domain of aggrecan has been noted in patients with AS, with 62 and 72% of patients having circulating and synovial CD4+ T cells with specificity for this antigen. The ability of the intervertebral disc to resist compression is in part due to the high aggrecan content whose hydration provides for osmotic pressure. The entheses, anterior uveal tract, aorta, and aortic valve all contain aggrecan or homologous proteins such as versican that provide for cyclic compression and relaxation. Immunity to aggrecan in BALB/c mice induces spondylitis and peripheral arthritis,⁴⁵ and the immunoreactive G1 domain is the major proteolytic degradation product of the proteoglycan. One explanation for these findings is that fibrocartilaginous enthesal microtrauma or local inflammatory processes secondary to infection in the immunogenetically predisposed patient may lead to an autoimmune reaction against newly exposed antigens that are also expressed at extraarticular sites typical of AS. Another potential explanation is that the G1 domain could induce antigen-specific tolerance with consequent suppression of the normal eradication mechanisms of inflammatory foreign material. A third possibility is that there may be no immunopathological role, with anti-G1 reactivity occurring as a secondary event after cartilage destruction caused by another primary process.

Aberrant Surface Heavy Chains. Although arthritogenic protein presentation may play a role in the pathogenesis of AS, this may not be the unique pathogenetic mechanism by which AS-associated HLA-B27 subtypes mediate disease. The T-cell-dependent development of colitis and arthritis in transgenic HLA-B27 rats is unchanged by CD8+ T-cell depletion, thus raising the question of whether other autoinflammatory pathways are invoked in this disease.⁸¹ Aberrant folding of HLA-B27 molecules can lead to unusual molecular conformations, and these nonconventional forms may trigger pathogenic autoinflammatory activity. In vitro folded HLA-B27 in the absence of β_2 -microglobulin had a propensity to form disulfide-bridged homodimers using an unpaired cysteine residue.¹ Although these dimers normally form when cell surface heavy chains lose β_2 -microglobulin during endosomal recycling, free monomeric HLA-B27 heavy chains have been observed from which surface dimerization is possible. The immunological consequence of these abnormal linkages has been studied using tetramerized dimers, with 1 receptor (KIR3DL2) specifically recognizing the supramolecular complex.^{58,59} Higher frequencies of KIR3DL2+ NK and CD4+ T cells have been found in patients with spondyloarthropathies.²⁸ More recently, the HLA-B27 homodimer recognition by KIR3DL2+ NK and CD4+ T cells has been found to be independent of the bound peptide sequence.⁶⁰ This nonconventional display of dimerized HLA-B27 can have the im-

munological consequence of eliciting unnecessary and destructive NK and CD4+ T-cell effector function autoreactivity, thereby triggering the disease process. The specific subtype of CD4+ T cells expanded among AS patients is CD28-, and this subtype has also been described as involved in other chronic inflammatory conditions such as Wegener granulomatosis, rheumatoid arthritis, and multiple sclerosis.³² These CD28- cells can sustain synovitis through the release of IFN γ and can also lyse target cells by releasing perforin.

Protein Misfolding. One fate of misfolded proteins is that they are degraded by the process of ERAD. If the capacity of cells to process the misfolded proteins through ERAD is exceeded, then these misfolded proteins may be deposited as intra- or extracellular aggregates. These aggregates are frequently toxic and can elicit nonspecific inflammatory reactions by mononuclear cells. The HLA-B*2705 polypeptide sequence has been shown to undergo slower protein folding and greater cytosolic accumulation than non-disease-prone HLA types.⁸⁴ There are various characteristics of the HLA-B*2705 polypeptide sequence that may contribute to such folding abnormalities. Abnormal tertiary protein folding with erroneous intramolecular disulfide bridging can occur, and this can be compounded by erroneous quaternary interactions of intermolecular covalent bond formation. Whereas the cell surface contains ~ 6% HLA-B27 homodimers, $\geq 25\%$ of heavy chains in the ER are disulfide bridged.³⁰ The cysteine-67 residue is implicated in this process, however, the identity of other residues in the binding groove influences appropriate folding. Specifically glutamate-45 is most detrimental to efficient protein folding, and lysine-70 increases the reactivity of the unpaired cysteine residue promoting supramolecular association.¹¹⁸

Another response to misfolded proteins is the UPR.⁵⁰ When the ER is stressed in cells expressing the aberrant protein, the cell can activate signaling cascades that attempt to deal with the altered condition and restore a favorable protein folding environment.⁶⁵ Macrophages derived from bone marrow of transgenic HLA-B27+ rats treated with IFN γ showed evidence of the UPR, whereas this response was absent in nontransgenic and non-disease-prone transgenic rats.¹¹⁰ This effect correlates with increased HLA-B27 expression, increased formation of misfolded heavy chains, and increased occupancy of the ER-resident chaperone protein, BiP. Heightened and prolonged interaction between HLA-B27 and BiP leads to simultaneous activation of ERAD and proinflammatory NF- κ B pathways. These are important responses of HLA-B27 biological inflammatory activity in the absence of specific immune recognition. In the same study, inflamed tissue from the gastrointestinal tract exhibited the same UPR activation; curiously this effect was not seen in other tissues such as the thymus and spleen, even in animals with active disease. Of further pathogenetic interest is that these transgenic rats undergoing the UPR exhibited synergistic induction of IFN β in response to lipopolysaccharide.¹⁰¹ The implications of this mechanism include activation of NK cells and macrophages, promotion of T-cell survival, and induction of dendritic cell maturation.^{68,108} This process is antigen-independent with activated macrophages secreting cytokines that augment inflammatory cell infiltration and MMPs that

can cause local tissue destruction. The latter may release antigens that can then become targets of a specific T-cell immune response. Although IFN β is also known to inhibit osteoclast activity,^{103,104} its role in the aberrant bone formation of AS remains unclear.

Enhanced Intracellular Microbial Survival. Enhanced intracellular microbial survival may also play a role in the pathogenesis of AS. Abnormal immune system activation or modulation can occur because of ineffective peptide loading onto HLA-B27, leading to excessive viral or intracellular bacterial proliferation and delayed antigenic peptide clearance. There is conflicting evidence to support this claim, however transfected monocytes do exhibit an enhanced survival of *Salmonella enteritidis*,⁸⁸ possibly because of a dysregulated p38 mitogen-associated protein kinase function. Conversely, clearance of *Salmonella* from intestinal epithelial cells⁹⁶ and synoviocytes⁵² seems to be unaffected under nonactivated conditions, but synovocyte coinubation with IFN γ did create a permissive environment for intracellular bacteria. This permissive environment may be the result of the decreased TNF α - and IFN γ -producing cells among patients with AS, with a decreased helper T-cell (Th1) response, leading to the impaired containment and eradication of the intracellular pathogens.⁹⁵

An alternate proposed mechanism conferring survival is the expansion of NK cells expressing the NK-inhibitory receptor carcinoembryonic antigen-cell adhesion molecule (CEACAM1). Natural killer cells from patients with AS and from healthy individuals reacted with HLA-B27, although the higher level of CEACAM1 expression among those with AS made these patients susceptible to ligand-mediated inhibition.⁴ The attenuated cytolytic activity of these cells may contribute to the prolonged survival of the antigenic, proinflammatory stimulus.

Evidence of Other Genetic Associations in AS

There is significant evidence to suggest that other genetic factors act to determine which of these patients have a heightened susceptibility to developing the disease, and family studies consistently show that HLA-B27+ patients who have a first-degree relative with AS have rates of disease development 6–16 times greater than those without such a family history.^{26,111} Twin studies further demonstrate this with AS concordance rates for monozygotic twins of 75%, for dizygotic twins of 12.5%, and for HLA-B27+ dizygotic twins of 27%.²⁴ In addition to incidence, Hamersma et al.⁴⁹ have assessed clinical severity using the Bath AS disease activity index and the Bath AS functional index and have reported heritability of 51 and 68% respectively.⁴⁹ Furthermore, the radiographic severity assessed with the Bath AS radiographic index has a heritability index of 0.62.²⁰ Because all of the patients in these studies were HLA-B27+, the observed variation in heritability reaffirms the role of non-HLA-B27 factors in clinical phenotype.²¹

Other MHC Associations

Given that 10% of Caucasian patients with AS are HLA-B27–, investigation into other MHC components has yielded information about other molecular associations of this disease. In both Caucasian and Taiwanese Chinese populations, carriage of HLA-B60 and HLA-B61 has been shown

to be increased in patients with AS, independent of HLA-B27 status.^{25,115} The peptide-binding motif of HLA-B60 is reportedly substantially different from HLA-B27;³⁶ however, the similar T-cell epitopes of both HLA-B60 and HLA-B61 with HLA-B27 supports a molecular mimicry mechanism.⁷¹

Various other MHC-related genes have been demonstrated to have an association with AS, but the putative mechanisms by which they are involved remain undefined. Class II MHC alleles such as HLA-DRB1*01 and DRB1*04 have also been associated with AS incidence, although not with disease severity or clinical manifestation.^{23,98} A study of 42 Japanese patients with AS reported that HLA-DR8 augments susceptibility to uveitis.⁸⁷ Low molecular weight proteasome (LMP) genes 2 and 7 encode subunits of a multicatalytic enzyme that degrades cytoplasmic proteins into oligopeptides that are eventually loaded onto MHC-I complexes. Several studies have reported that specific LMP2¹¹² and LMP7⁴² alleles increase susceptibility to AS and the likelihood of developing the extraarticular symptoms of AAU.^{77,78} One study in 150 Mexican patients suggests an association of several HSP70 gene polymorphisms with AS.¹¹³ Although HSP70-2 and HSP70-hom were found to have a significant association with AS in patients both positive and negative for HLA-B27, such findings were not replicated in studies of Finnish or Spanish patients.^{41,117} There is currently no proposed mechanism linking HSP allelic differences with autoimmune disease.

Non-MHC Genetic Associations

Genomic screening has yielded evidence for non-MHC genetic contributions to the development of AS. Whole-genome screening in 185 families with AS and 255 affected sibling pairs revealed that the strongest linkage was found on chromosome arm 16q (logarithm of the odds score of 4.7).⁶⁷ Other regions with moderate evidence of linkage exist on chromosomes 3, 10, and 19, and nominal linkage was demonstrated on chromosome arms 2q and 22q. Other authors have described the specific non-MHC genes associated with AS to include the IL-1 gene complex, cytochrome P450 2D6 (CYP2D6), and TGF β .²¹

The IL-1 complex encodes proinflammatory cytokines IL-1 α and IL-1 β on chromosome 2. There is widespread association with AS across the IL-1 gene cluster. The most implicated region encodes IL-1 α , although the specific variants that cause disease remain unidentified.¹⁰⁹ Interleukin-1 receptor antagonist (IL-1Ra) shares some sequence homology as a competitive cytokine receptor antagonist, and various single nucleotide polymorphisms are also associated with AS.⁷⁶ Of mechanistic interest is that various clinical studies demonstrate that inhibition of IL-1 activity by recombinant human IL-1Ra in patients with nonsteroidal antiinflammatory drug-resistant disease leads to limited improvement in both clinical⁴⁸ and radiographic¹⁰⁵ evaluations.

The CYP2D6 gene, encoded at 22q13, is a mixed-function oxidase implicated in the metabolism of xenobiotics. Loss of function mutations that decrease oxidative drug metabolism occur in 5–10% of Caucasians, with a lower incidence in other ethnic groups such as Asians (2%). Such mutations have been reported by several authors to be asso-

ciated with AS,^{10,22} with a hypothesized mechanism of reduced metabolism of the natural antigens or toxins that may trigger the disease onset.

Involvement of TGFβ has been suspected based on its location on chromosome arm 19q, a region with moderate linkage in whole-genome screening, as well as on its functional involvement in inflammation, tissue fibrosis, and bone remodeling. Two single nucleotide polymorphisms that have received attention include TGFBI+915 and TGFBI+1632. Both polymorphisms have been associated with disease incidence, and the former is also reported to yield higher circulating levels of this anabolic protein.⁵⁴ High circulating TGFβ levels have also been observed by other groups,⁵¹ but the mechanism and impact of this cytokine imbalance remain unclear.

Cytokine and Enzyme Effectors

The immunopathogenesis of AS is suspected to involve downstream upregulation of proinflammatory cytokines. Tumor necrosis factor-α is consistently found to be more highly expressed in patients with AS than in healthy individuals,^{5,47} and there is evidence that anti-TNFα therapies can effectively improve both clinical and laboratory disease parameters.^{12,15–17,116} Heightened serum levels of IL-6 and soluble IL-2 receptor have been found in patients with AS, and the latter was shown to have a positive association with fatigue and pain scores and with the Bath AS Functional and Metrology Indices.⁵

Many cell lines produce MMPs in response to proinflammatory cytokines such as TNFα and IL-1, and these Zinc-dependent endopeptidases normally play an important role in the degradation of the extracellular matrix. Excessive proteolysis in the inflamed joint can result from a pathological imbalance between MMPs and their inhibitors, and a higher expression of MMP-3 has been found among patients with AS,²⁹ with quantitative levels positively correlating with both the Bath AS Disease Activity and Functional Indices. Matrix metalloproteinase-3 has been advocated as a biomarker of disease activity that is more accurate than erythrocyte sedimentation rate or C-reactive protein levels. The putative roles of MMP-3 include the primary digestion of extracellular matrix components and enzymatic activation of various other pro-MMPs to induce further proteolytic activity.¹¹⁴

Environmental Triggers

Although various other spondyloarthropathies have been linked to infectious origins, the role of microbial pathogenesis in the development of AS has remained undefined. Animal studies have shown that the genetically susceptible HLA-B27-transgenic rats only exhibit the disease phenotype on exposure to a pathogen,¹⁰⁷ but less definitive evidence exists among human patients, and no organism has been identified in synovial fluid and sacroiliac joints.¹⁸ Circumstantial evidence of microbial involvement has been borne out in demographic studies suggesting higher rates of enteric infection among patients with AS and undifferentiated spondyloarthropathy who have active disease.⁸⁰ There are specific bacterial agents with an established relation-

ship to HLA-B27, and these include *Campylobacter*, *Chlamydia*, *Shigella*, *Salmonella*, *Yersinia*, and *Klebsiella*.^{33,61,69,102}

Of specific interest is *Klebsiella pneumoniae* infection as a triggering or perpetuating factor in the pathogenesis of AS through the mechanism of molecular mimicry. There is significant sequence homology for a hexapeptide epitope (QTDRED) in HLA-B27 and *Klebsiella* nitrogenase reductase.⁵³ There is a further tetrapeptide homology for HLA-B27 and the *Klebsiella* pullulanase Pul-D domain, and tripeptide homology for Types I, III, and IV collagens and *Klebsiella* pullulanase Pul-A domain.³⁷ Antibodies to such epitopes are cross-reactive with synovial tissues from HLA-B27+ patients.⁹⁰ There are several lines of evidence that suggest that *Klebsiella*, and not other microbes, is responsible for AS.⁹⁰ *Klebsiella* microbes have been isolated in more than 70% of AS patients with AAU,³⁴ and elevated levels of anti-*Klebsiella* antibodies have also been detected in these patients.⁹⁷ In addition, patients with biochemically inactive AS, as assessed by normal erythrocyte sedimentation rate and C-reactive protein levels, do not have elevated antibody titers against *Klebsiella*.¹⁰⁶ The antibodies against *Klebsiella* produced in the gut could bind to cross-reactive antigens in the nearby joints and via the Batson plexus to the lumbar spine. The increased prevalence of AS in young adult males may result from a higher starch intake and consequent increase in *Klebsiella* growth in the gut.² The site at which the triggering infection occurs is unclear, but the presence of histological signs of inflammation⁸⁶ and increased gut permeability⁷⁹ in patients with AS suggests an overt or occult enteric infection.

Pathological Features of AS

Inflammation in AS occurs primarily at the sacroiliac joints, but can involve entheses, vertebral bodies adjacent to the intervertebral discs, and peripheral joint synovium. The extraarticular pathological features include involvement of the ophthalmic, cardiovascular, pulmonary, gastrointestinal, and renal systems, and are summarized in

TABLE 3
Extraarticular features of AS

Organ System	Manifestations
ophthalmic cardiovascular	AAU
	aortitis aortic valve insufficiency thickening of aortic valve leaflets aortic valve insufficiency subaortic fibrosis conduction abnormalities mitral valve insufficiency left ventricular dysfunction
gastrointestinal neurological	inflammatory bowel disease myelopathy & radiculopathy cauda equina syndrome vertebrobasilar insufficiency peripheral neuropathy
pulmonary	interstitial fibrosis restrictive thoracopathy
renal	amyloidosis
	immunoglobulin A nephropathy

Table 3. In the present study, our discussion is limited to addressing the spinal and articular pathological characteristics of AS.

In AS, the apophyseal, costovertebral, and sacroiliac joints of the axial skeleton are most typically affected and may ultimately become ankylosed. The enthesitis that is typical of AS occurs at fibrocartilaginous sites, with the enthesis originating at the cartilage and attaching at the epiphyses or apophyses. Affected enthesal fibrocartilage structures are generally exposed to significant shear stress and microtrauma, and it is suspected that this pathomechanism is necessary to expose tissue antigens to circulating lymphocytes. The accompanying osteitis is not haphazard, but rather follows the lines of mechanical stress in the bone.⁸² In genetically susceptible individuals, such exposed antigens can elicit autoinflammatory and autoimmune reactions. The more extensive oligoclonal expansion of CD8+ T cells that occurs in patients with AS compared with healthy HLA-B27+ individuals further supports the antigen-specific pathological mechanism of this disease.^{7,9}

Histopathological changes are of a fibrocartilaginous inflammatory reaction including hyperosteoclastic, erosive lesions infiltrating the underlying bone marrow.⁶⁶ Further changes include an increased quantity of enthesis fibrocartilage, enthesophytes formed by endochondral ossification, fissures and cracks, and extensive granulation tissue. In humans, enthesophytes range from small lesions at the Achilles and patellar tendons and plantar aponeurosis, to larger spurs forming complete ossification between adjacent vertebral segments with the classic “bamboo spine” appearance. The steps by which these enthesophytes form remain elusive, but a necessary antecedent step includes fibrocartilage cell proliferation. The results of animal studies have demonstrated that capillary invasion from underlying bone marrow creates tunnels that are eventually filled with bone.⁸ Ankylosis of the axial spine tends to begin in the lumbar area and ascends. Cervical spine involvement with development of neck pain occurs as a function of disease duration, with 20% affected at 5 years and 70% at 20 years.³⁵ The osseous extension or ankylosis is thought to be initiated by the mechanical forces of muscle pulling, and mediated by the interaction between bone morphogenetic proteins, activins, and TGF β . The cellular infiltrate initially comprises CD68+ macrophages that yield to predominantly CD8+ T cells in patients with established disease.^{66,83}

Sacroiliitis is characterized by synovitis and myxoid bone marrow with formation of pannus and granulation tissue. These processes erode, destroy, and replace articular cartilage and subchondral bone. Paraarticular bone is also thickened by the stimulated osteoblastic activity. The combination of concomitant synovitis, subchondral bone marrow inflammation, and cartilage destruction and regeneration has implicated sacroiliitis in AS as more than simply an extension of the above enthesitis.⁴³ The sacroiliac joint inflammation in AS has a predilection for the iliac side of the joint, and this may be due to the greater fibrocartilaginous lining and the greater shear stresses experienced on that side.⁵⁶ Neovascularization is also common, along with proliferating fibroblasts and molecular upregulation of TNF α and TGF β transcripts. Bone destruction is mediated mainly by CD68+ macrophages and CD4+ T cells, followed by endochondral ossification and bone ankylosis.¹³ The cellular infiltrate is composed of CD68+ macrophages, CD4+ T

cells, B cells, and NK cells.¹¹ Although gluteal region discomfort often implicates a sacroiliac joint pathological entity, discomfort in this region can also be complicated by a spasm of the piriformis muscle and compression of the sciatic nerve.

In AS, entheses of the lower limbs are more commonly involved than those of the upper limb. The heel is the most frequent location of enthesitis in AS, with inflammation at the plantar fascia eroding the calcaneus and causing consequent periostitis and bone spur formation. Asymmetrical, oligoarticular peripheral arthritis also develops among most AS patients during the course of their disease. The late changes of ankylosis and osteoarthritis can lead to loss of function and total joint arthroplasty in 8–15% of patients.^{46,93}

Despite the increased ankylosis of joints, patients with AS commonly have decreased bone mineral density with consequent presentations of nontraumatic fractures in young, male patients. Osteoporosis of the femoral neck occurs in one third of patients, with osteopenia afflicting a further 41%. The lower bone mineral density in these patients seems related to the activity and severity of the underlying inflammatory process,⁵⁷ although the mechanism remains unclear. Heightened levels of the soluble receptor activator of N- κ B ligand promote osteoclast activity despite normal osteoprotegerin levels that antagonize osteoclast activity, creating a homeostatic perturbation towards bone resorption. Other causes of osteoporosis beyond the inflammatory osteoclast activation in this population include corticosteroid use, immobility, and hormonal imbalance.⁸⁵

Appel and coworkers³ have established a correlation of histopathological features and magnetic resonance imaging evaluations in 8 patients with AS. The chosen sequence was T2-weighted images with fat saturation in which water appears to have a high intensity. Bone marrow edema of the lumbar spine zygapophyseal joints detected by this examination correlates with interstitial edema on histological examination but not with the underlying inflammatory cell infiltration. The regression of the edemic changes seen on MR imaging has been demonstrated as early as 6 weeks after treatment with disease-modifying anti-TNF α therapy.⁹⁴

Conclusions

The clinical manifestations of AS are believed to result from a combination of an immunogenetic predisposition and a triggering biomechanical, inflammatory, or infectious event that leads to disease. The precise sequence of the immune activation and consequent histopathological characteristics has remained undefined. Strong associations with the presence of HLA-B27 could result from various molecular abnormalities ranging from the presentation of novel arthritogenic peptides to an abnormal autoimmune stimulation to an anomalous microbial tolerance. The role of an antecedent infection in the onset of AS has not been as clearly established as for other seronegative spondyloarthropathies, although significant evidence of an immunoreactivity against *Klebsiella* in this patient population may represent a primary pathogenic step. Furthermore, a biomechanical triggering event can initiate either exposure to previously immune-sequestered autoantigens or provide

a route for bacterial seeding to initiate inflammation. A better comprehension of the sequence of molecular events that actualize AS will facilitate the development of more specific and targeted therapies.

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