

## The Involvement of HLA-B27 in Ankylosing Spondylitis

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**Abstract:** Ankylosing spondylitis is a chronic inflammatory disease that has been linked to the human leukocyte antigen class I allele HLA-B27. More than 90% of patients with ankylosing spondylitis have the HLA-B27 allele, but only 1% of people with HLA-B27 develop the disease. This suggests that disease progression is triggered by an environmental factor in genetically predisposed individuals. At this time, the direct relationship of HLA-B27 with ankylosing spondylitis is unknown. Studies with transgenic rodents suggest that free heavy chains bind together through a cysteine at position 67 of the  $\alpha 1$  domain to form a homodimer that mimics a HLA class II molecule, allowing it bind peptides up to 33 amino acids long, and to activate CD4<sup>+</sup> T-cells. The presentation of an “arthritogenic peptide” and subsequent activation of CD4<sup>+</sup> T-cells could be the inciting event that leads to disease presentation.<sup>2,4,12</sup>

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Ankylosing spondylitis (AS) is a chronic spondyloarthropathy characterized by inflammation of articular surfaces, most notably the sacroiliac joints, joints of the axial skeleton, and paravertebral soft tissues.<sup>10,19</sup> Disease progression may lead to bony ankylosis of the joint at the attachment site of ligaments and tendons. Inflammation at the joint leads to subsequent erosion of the underlying bone. The paravertebral soft tissues are then invaded by granulation tissue, causing replacement of soft tissue with bony tissue. Subsequent calcification and ossification of the new bony tissue causes ankylosis of the joint.<sup>19</sup> In the intervertebral disk, the new bone is formed beginning within the outer layers of the annulus fibrosis. Syndesmophytes, or bony bridges between adjacent vertebrae are then

formed, restricting mobility of the spine.<sup>7,10,13,19,20,22</sup>

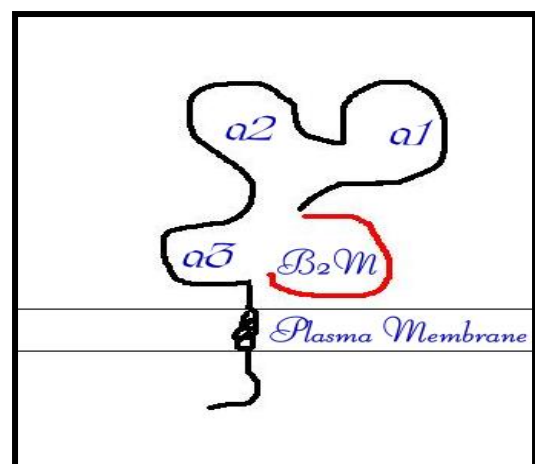
Ankylosing spondylitis is a disease that predominately affects males and usually presents in young adulthood. It usually begins with unilateral pain at the sacroiliac joint, progressing to bilateral pain that radiates down the legs, and up the spine, eventually affecting the rib cage. In severe cases, chest expansion becomes impaired, and the cervical spine becomes ankylosed, restricting movement of the head and neck. Eventually the spine becomes completely rigid, causing loss of motion in all planes and loss of the normal kyphotic and lordotic curvatures. Criteria for diagnosing AS include bilateral sacroiliitis with low back pain and stiffness for more than 3 months, limited chest expansion, limited motion of the lumbar spine in anterior flexion, lateral

flexion, and extension, and pain and stiffness in the thoracic region. Usually the pain associated with the disease improves with exercise, but worsens during periods of inactivity, such as during sleep. Nonskeletal problems that may occur in conjunction with AS include iritis, uveitis, pulmonary fibrosis, inflammatory bowel disease, and aortitis.<sup>7,10,13,19,20,22</sup>

The disease ankylosing spondylitis has been linked to the human leukocyte antigen (HLA) class I allele HLA-B27 (see Figure 1), and the association of HLA-B27 with AS is the strongest for any HLA locus.<sup>1</sup> Class I HLA molecules are present on almost all nucleated cells in the body, except neurons and striated muscle cells.<sup>12</sup> Studies have shown that 90-94% of AS sufferers have HLA-B27, while 5-9% of the general population have the HLA-B27 allele.<sup>5,10,13</sup> The disease is most likely triggered in genetically predisposed individuals by an environmental factor, since only 1% of the people with the HLA-B27 allele develop ankylosing spondylitis. The exact mechanism of triggering the disease is unknown at this time, but several theories have been proposed to explain the involvement of HLA-B27 in the disease.<sup>19</sup>

HLA molecules are synthesized in the cell through the secretory pathway, beginning in the rough endoplasmic reticulum. The nascent class I heavy chains are bound by the membrane-bound chaperone, calnexin, which prevents improper folding in the RER. When the heavy chain binds with  $\beta_2$ -microglobulin ( $\beta_2m$ ), calnexin dissociates, and the new heterodimer then binds another chaperone, calreticulin. Peptide loading of the heterodimer is dependent on the transporter associated with antigen processing 1 (TAP1), and its associated protein, tapasin. Binding of tapasin with the heterodimer and with TAP1 allows a peptide of suitable size to be inserted into the HLA molecule.

Peptides, usually 8-10 amino acids long, resulting from the degradation of cytosolic proteins by a proteasome, are transported through the TAP complex, and are inserted into the HLA molecule. The heterodimer then folds properly, and continues along the secretory pathway for expression on the extracellular surface of the cell membrane. Since class I HLA molecules display endogenous antigens, self- proteins and foreign proteins resulting from an infection are incorporated into the binding pocket of HLA-B27. CD8<sup>+</sup> cytotoxic T lymphocytes recognize class I HLA molecules and cause the destruction of infected cells when foreign peptides are displayed. During T-cell maturation in the thymus, autoreactive T lymphocytes are destroyed in a process known as “clonal deletion,” so self-proteins do not usually trigger an immune attack. However, antigens that are present only in certain tissues cannot act in T-cell maturation, and thus autoreactive T cells can exist if they are not destroyed by other mechanisms.<sup>17</sup>



**Figure 1: Class I HLA molecule.** The molecule is a polypeptide heterodimer consisting of a transmembrane heavy chain noncovalently associated with  $\beta_2$  microglobulin. The peptide binding cleft exists between the  $\alpha_1$  and  $\alpha_2$  domains.<sup>14</sup> (Adapted from Janeway, et. al., 2001)

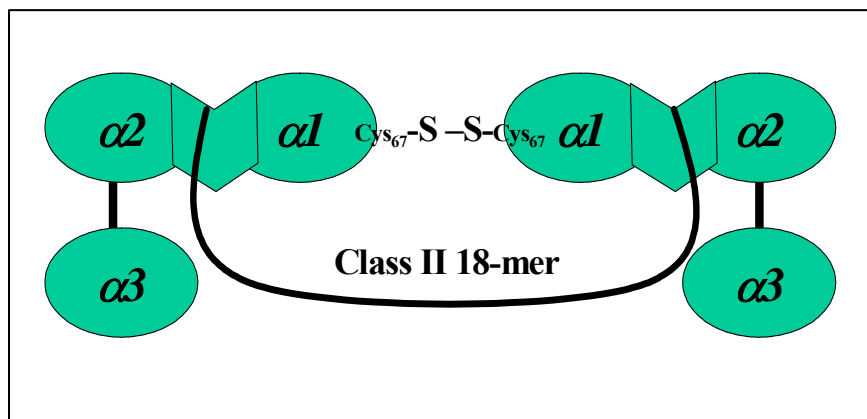
One theory of the involvement of HLA-B27 in AS states that antibodies against pathogens such as *Klebsiella pneumoniae* trigger immune responses against autoantigens in a type of “molecular mimicry.” Autoimmunity may result when class II HLA molecules present HLA-B27 peptides to CD4<sup>+</sup> T-cells that have been activated by bacterial antigen during infection.<sup>1</sup> Epidemiological studies have shown a correlation between bowel infections of *Klebsiella* preceding the onset of AS. *K. pneumoniae* was isolated from AS patients with greater frequency during active phases of the disease, and relapses were preceded by its isolation from patients’ fecal samples. Amino acid homology has been demonstrated between the HLA-B27 molecule and the *Klebsiella pneumoniae* nitrogenase reductase enzyme. Both molecules contain the amino acid sequence QTDRED, and patients with AS exhibit increased levels of antibodies to the *Klebsiella pneumoniae* nitrogenase reductase enzyme.<sup>11</sup> Studies show that anti-HLA-B27 antibodies also cross-reacted with *Salmonella*, *Shigella*, and *Yersinia enterocolitica*, which are organisms that can trigger reactive arthritis in individuals with HLA-B27.<sup>3,17,25</sup> Furthermore, inflammatory stages of the disease were associated with increases in serum IgA, which would suggest that a pathogenic microbe was acting on a mucosal surface.<sup>11</sup> However, cross-reactive antibodies present in both patients with ankylosing spondylitis and controls without disease, gives evidence against autoimmune molecular mimicry.<sup>22,25</sup>

The murine animal model for ankylosing spondylitis is ankylosing enthesopathy (ANKET), and mice reared in germ-free conditions do not develop ankylosing enthesopathy. The disease mirrors human ankylosing spondylitis in

affecting the joint entheses, and preponderance among young male mice. The molecular mimicry theory is supported by the fact that some mice reared in germ-free conditions then conventionalized developed ankylosing enthesopathy, but none of the mice kept in germ free conditions developed the disease. The fact that one ex-germ free mouse developed the disease despite being fed a sterilized diet would imply that neither killed bacteria nor microbial compounds in the food induce the disease.<sup>21</sup> Thus, it can be assumed that exposure to and/or infection with live bacteria may be the inciting event in development of the disease.

The strongest evidence of the involvement of HLA-B27 in AS came from studies done with mice and rats that had been given HLA-B27 as a transgene.<sup>5,6,17</sup> They developed diseases such as ankylosing enthesopathy, and rats with a high copy number of HLA-B\*2705 developed axial and peripheral arthritis, gut inflammation, and lesions on the skin. Rats and mice that were kept in sterile environments did not develop joint disease or gut inflammation. Development of disease was dependent on the presence of gut bacteria and a high copy number of HLA-B27 in cells of bone-marrow lineage.<sup>24</sup>

HLA-B27 has an unpaired cysteine residue at position 67 of its  $\alpha_1$ -domain, and this seems essential for disease progression because rats transgenic for a mutated form of HLA-B27 without Cys at position 67 have reduced incidence of the disease.<sup>5,24</sup> It is possible that the cysteine could bind to other molecules, creating an “altered self” through a disulfide bond.<sup>3</sup> It has been shown that HLA-B27 can form homodimers *in vivo*<sup>4</sup> of the heavy chain through the cysteine residue of position 67 of the  $\alpha_1$ -domain (See Figure 2). This homodimer structure could bind molecules longer than the usual 8 to 10



**Figure 2: Free HLA-B27 heavy chains can form a disulfide-bonded homodimer through a cysteine residue at position 67 of the  $\alpha 1$  domain. These homodimers can form in the absence of  $\beta_2$ -microglobulin, and can bind peptides longer than the usual 8-10 amino acids.<sup>1,4,5</sup>**

**Homodimer formation**

may explain why  $\beta_2$ -microglobulin-deficient mice exhibit signs of the spondyloarthropathies when given HLA-B27 as a transgene.<sup>2</sup>

amino acids and present them to CD4<sup>+</sup> T-cells, initiating the disease,<sup>2,4,5</sup> and in fact, peptides of up to 33 amino acids long have been eluted from HLA-B27 molecules.<sup>1</sup>

There is evidence implicating both CD8<sup>+</sup> and CD4<sup>+</sup> T-cells in disease pathogenesis, but it is thought that CD4<sup>+</sup> T-cells play a greater role, because transgenic mice with extremely low levels of CD8<sup>+</sup> T-cells develop disease.<sup>6</sup> Transgenic studies have shown that the most effective inducer of disease was transferring purified CD4<sup>+</sup> T-cells from arthritic transgenic rats into HLA-B27 recipient rats.<sup>17</sup> Transgenic mice only developed AS when they were also transgenic for human  $\beta_2$ m; those with murine  $\beta_2$ m did not develop symptoms of the disease.<sup>5</sup> During synthesis of HLA class I molecules, the heavy chains associate with  $\beta_2$ m and endogenous peptides before being trafficked to the cell surface. HLA-B27 is unusual compared to the other class I HLA molecules in that it is slow to fold and associate with  $\beta_2$ m.<sup>18</sup> HLA-B27 has been shown to misfold both in the presence and absence of TAP and  $\beta_2$ m,<sup>8</sup> but with a greater frequency in the absence of  $\beta_2$ m.<sup>18</sup> Thus, homodimers and free heavy chains are formed with greater frequency than with other class I alleles, and can be trafficked to the cell surface.<sup>9,16</sup> Homodimer formation and presentation of “arthritogenic peptides” could explain the incidence of disease pathogenesis in  $\beta_2$ m-deficient mice.<sup>2</sup>

Transgenic studies also gave evidence that disease pathogenesis is not dependent on presentation of an “arthritogenic peptide” or HLA-B27 derived peptides in class II molecules. Transgenic mice expressing HLA-B27 but lacking endogenous  $\beta_2$ m, were bred with knockout mice lacking the class II knockout gene A beta. The occurrence of disease in the mice lacking the murine class II molecules H2-A and H2-E indicated that disease development is not dependent on presentation by class II molecules, but is directly related to the presence of HLA-B27 heavy chains.<sup>15</sup>

Homodimer formation *in vivo* that would present peptides to class II HLA molecules would depend on the presence of CD4<sup>+</sup> T-cells capable of binding to HLA-B27 molecules. Boyle, et al, isolated CD4<sup>+</sup> T-cells that recognize HLA-B27 molecules in cell cultures that are deficient in HLA class II molecules. The CD4<sup>+</sup> T-cells recognize HLA-B27 transfected cells that exhibited different forms of HLA-B27, including the heavy chain homodimers, free heavy chains, and normal heterodimers. They also showed that binding of the CD4<sup>+</sup> T-cells was inhibited by the antibody mAb ME1, which is specific for HLA-B27.<sup>6</sup> Thus, clear evidence was presented that

HLA-B27 can be recognized by CD4<sup>+</sup> T-cells. This activation of CD4<sup>+</sup> T-cells by HLA-B27 could explain why bacteria-specific CD4<sup>+</sup> T cells are sometimes found in the diseased tissues of patients with AS.<sup>6,17</sup>

**Conclusion:** It is unclear exactly what role HLA-B27 has in provoking the disease ankylosing spondylitis, and other alleles may be acting in concert with B27 to elicit the disease. Despite the fact that CD8<sup>+</sup> and CD4<sup>+</sup> T-cells are found in the synovial fluid of AS patients, it is probable that CD4<sup>+</sup> T-cells play a dominant role in disease initiation.<sup>4,17</sup> The fact that unusually long peptides can be presented by the HLA-B27 homodimers to CD4<sup>+</sup> T-cells would correlate the theories of an “arthritogenic peptide” with misfolding of the B27 molecule. The presentation of peptides by HLA-B27 to CD4<sup>+</sup> T-cells would explain the inflammatory characteristics of AS, as well as the presence of cross-reactive antibodies in patients with AS.<sup>17</sup> Future studies would involve proving the correlation of HLA-B27-derived peptides with activation of CD4<sup>+</sup> T-cells, and explaining why disease progression occurs primarily in the joints of the axial skeleton.

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