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1. Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease with potential disabling outcomes. Clinically, patients presented with inflammatory lower back pain, enthesis and alternated buttock pain (van der Linden & van der Heijde, 1998). Bernard Corner (1666-1698) was the first physician who published the clinical features of AS in his medical thesis (Baker & Weisman, 2006). In the late 19th century, 3 independent physicians: Marie, Strupell, and Bechterew were able to describe the specific radiographic change in those patients by the help of the invention of radiology (Bywaters, 1983). But, still clinically, the boundary between AS and rheumatoid arthritis was unclear. Thanks to the discovery of rheumatoid factor which was strongly associated with rheumatoid arthritis, the distinction between these two arthritides became crystal clear. In those patients with inflammatory lower back pain and seronegative for rheumatoid factor, the diagnosis of ankylosing spondylitis became more popular in the early 1960 (Zeider et al., 2011). In 1963, American Rheumatism Association proposed a new nomenclature and classification for the rheumatic diseases. In this new edition, AS was specified as a complete different disease entity from rheumatoid arthritis (Blumberg et al., 1964). In addition to those different clinical characteristics, such as bone proliferation in enthesis site and sacroiliitis, in AS patients from those of patients with rheumatoid arthritis, AS is also known for its high association with HLA-B27. It has been known for more than 30 years since this association was discovered at 1973 (Schlosstein, 1973; Brewerton et al., 1973), although afterward, researchers found several HLA antigens were associated with other diseases (Invernizzi, 2011; McElroy, 2011; Piga, 2011), the strongest of any HLA antigens associated with human disease is HLA-B27 molecule. Hence, the roles of HLA-B27 in the pathogenesis and clinical manifestation of ankylosing spondylitis were among most frequent discussed topics in the past three decades.

2. Structure, subtypes and epidemiology of HLA-B27

HLA-B27 is one of the HLA class I molecules which are highly polymorphic and plays major role in protective immunity against intracellular parasites including virus and bacteria (Bjorkman et al., 1987). Traditionally, HLA class I molecule is considered to present peptide antigens to cytotoxic (CD8+) T cells. X-ray crystallographic studies revealed that extracellular structure of heavy chain of class I molecule contained three components: α-1, α-2 and α-3 domains. α-1 and α-2 together with a β pleated intervening sequence to form a peptide
binding groove. α-3 domain is the membrane-proximal portion of the heavy chain which interact with CD8 of cytotoxic T cells. Besides binding peptides, class I molecule must associate non-covalently with beta2-microglobulin to form the tri-molecule complex on the cell surface. In lack of any one of these molecules, the molecular stability of this tri-molecule complex will be weak and easy to be degraded (Natarajan et al., 1999; Madden et al., 1991). Through their different amino acid compositions at binding groove, different HLA class I antigens has their own specific selectivity of binding peptides (Madden et al., 1992). In addition, to the selectivity of binding peptides, differences in the amino acid composition also influence the strength of association between heavy chain and beta2-microglobulin. (David, 1997).

HLA-B27 is a unique HLA class I molecule, not only because of its high association with AS but also has characteristically different amino acid composition from other class I molecules. In brief, there are two important characteristic structures which are different from others: the presence of B pocket and the free thiol group of Cys67 (Madden, 1995; Powis et al, 2009). In the presence of B pocket in the binding groove, B27 anchoring peptides had a very specific P2 residue: arginine. Free thiol Cys67 residue made B27 molecule easy to form homodimer in the extracellular domain which has great impact on its physiological role (Allen et al, 1999). There is an astonishing distribution of HLA-B27 gene among world population, with highest prevalence in northern territory of the earth, Eskimos and Native American in the circumpolar area and north Canada were known for their high carrier rate and some of the world’s highest prevalence rates of spondyloarthopathies are described in these groups (Peschken & Esdaile, 1999; Boyer et al., 1997). It was shown that the distribution of HLA-B27 had a tendency of a decreasing north-south gradient of prevalence and was speculated that the peculiar geographic distribution of HLA-B27 might reflect a genetic selection for better survival from microbial infection (Piazza et al., 1980) (Figure 1).

Fig. 1. The structure of HLA-B27 molecule. E_{45} and C_{67} are shared between all predisposing alleles. The presence of unpaired C_{67} made B27 molecule easy to form homodimers. In addition, the presence of H_{9} in the floor of β pleated sheet is critical for the stability of the heavy chain/β2-microglobulin complex.
Till July 2011, 82 HLA-B27 subtypes were described based on nucleotide differences (International IMunoGene Tics information system [IMGT], 2011). Most nucleotide changes locate at exons 2 and 3 which encode the α-1 and α-2 domains. HLA-B*27:05 is the most prevalent subtype and present in almost every population in the world. It was thought that HLA-B*27:05 was the ancestor subtype, all other subtypes could have evolved from HLA-B*27:05 by point mutation (B*27:03), reciprocal recombination (B*27:07, B*27:09) and gene conversion (B*27:01, B*27:02, B*27:04, B*27:06). Following the ethnic migration and genetic evolution, HLA-B27 evolved into three ancestral pathways. Each pathway developed into a specific pattern. The first pattern was characterized by amino acid substitutions in the α-1 domain. HLA-B*27:02 was the most frequent allele, followed by HLA-B*27:03. This pattern is found largely in Africa, Middle Eastern and European groups. The second pattern contains a constant substitution at α-1 domain and variable substitutions at α-2 domain. HLA-B27:04 was the most prevalent subtype. This pattern is largely found in Eastern Asian such as Chinese, Thai and Korean. The third pattern contains a similar α-1 domain as HLA-B*27:05 and variable substitution at α-2 domain. In which, HLA-B*27:07 is the most prevalent subtype. This pattern is largely found in Middle East, but also in Turkey and Greece (Reveille & Maganti, 2009) (Figure 2).

Fig. 2. Phylogenetic trees for the most common HLA-B27 subtypes (Adapted and modified from Blanco-Gelaz et al., 2001).

It is interesting to note that not all subtypes are associated with AS. In addition to B*27:05, most alleles such as B*27:01, B*27:02, B*27:03, B*27:04, B*27:10, B*27:13, B*27:14, B*27:15 are documented to be associated with ankylosing spondylitis (Taurog, 2007). In Chinese
population, B*27:04 seemed to play major role in the pathogenesis of AS. Meta-analysis results showed a positive association between B*27:04 and susceptibility to AS in Han population (Zang et al., 2011). Also in the Taiwanese population, susceptibility to AS was determined by the presence of HLA-B*27:04 (Hou et al., 2007). In contrast, two subtypes, B*27:06 and B*27:09 have been reported not to be associated with AS and even considered to play a protective role. B*27:06 is a common subtype in countries such as Indonesia, Singapore and Thailand. In Singapore Chinese, B*27:06 had a significant negative association with AS (Ren et al., 1997). The same result also was reported from Taiwanese patients (Chen et al., 2002). Similarly, B*27:09, a rare subtype primarily found in Sardinia island and southern Italy, was found to have negative association with AS (Fiorillo et al., 2003). In addition to B*27:06 and B*27:09, other HLA-B27 alleles such as B*27:08 in Venezuela and B*27:07 in Cyprus were claimed not to be associated with AS (Armas et al., 1999). But these results are not universal, in other populations the same alleles have been found in AS patients (Cipriani et al., 2003; Varnavidou-Nicolaidou et al., 2004; Paladini et al., 2005). (Table 1)

<table>
<thead>
<tr>
<th>B27 subtype</th>
<th>Ethnic distribution</th>
<th>Association with AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>B*27:05</td>
<td>Most ethnic groups</td>
<td>+</td>
</tr>
<tr>
<td>B*27:02</td>
<td>Caucasians, Central American, American Indians</td>
<td>+</td>
</tr>
<tr>
<td>B*27:03</td>
<td>Africans</td>
<td>+</td>
</tr>
<tr>
<td>B*27:04</td>
<td>Asians</td>
<td>+</td>
</tr>
<tr>
<td>B*27:06</td>
<td>Asians</td>
<td>–</td>
</tr>
<tr>
<td>B*27:07</td>
<td>Caucasians, Cyprus, middle east</td>
<td>+</td>
</tr>
<tr>
<td>B*27:08</td>
<td>Caucasians, Central Americans</td>
<td>+</td>
</tr>
<tr>
<td>B*27:09</td>
<td>Sardinian, Italy</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 1. The ethnic distribution and ankylosing spondylitis association of most frequent subtypes of HLA-B27.

3. Hypotheses of the role of HLA-B27 in the pathogenesis of ankylosing spondylitis

Since the discovery of the high association of HLA-B27 with AS, models have been proposed to explain the role of B27 in the pathogenesis of AS. Several aspects of research were made including epidemiology studies, analysis of molecular structure, transgenic animal models and analysis of environmental factors. Using these tools, during the past three decades, several hypotheses had been raised successfully to explain some aspects of this association, but still, like the story of blind men and elephant, each hypothesis touch tangentially different appendages of the animal. The correct description fit all aspects of the elephant remained unsolved.

In most population, B27 carry-rate was more than 80% in patients with ankylosing spondylitis. However, B27 carry-rate in patients with ankylosing spondylitis was less than 50% in some areas. For example, among African Americans, 50% of patients with ankylosing spondylitis possess HLA-B27 (Akkoc & Khan, 2006). Focus had been put on the effect and level of gene expression of B27 on disease presentation. It was reported that disease
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developed earlier in patients who were HLA-B27+ than those HLA-B27- (Wu et al., 2009). B-27+ patients had higher incidence of anterior uveitis and hip joint involvement (Khan et al., 1977; Feldtkeller et al., 2003). The level of HLA-B27 mRNA had been claimed to be correlated with clinical disease activity in Chinese patients (Liu, 2006). It is interesting to find that in animal study, the copy number of HLA-B27 genes seemed to be a critical factor in determining the expression of a arthritis phenotype (Mammer et al, 1990). However, the effect of B27 homozygosity on the risk of disease development was controversial (van der Linden et al., 1984; Kim et al., 2009; Jaakkola et al., 2006). On the other hand, only a small percentage of B27-carriers developed AS. Twin studies suggest that susceptibility to AS is more than 90% inherited. HLA-B27 accounts for more than 50% of this inheritance (Brown et al., 2000; Brown et al., 1997). Other genes must be involved in the disease development. A genome-wide study identified several other candidate genes such as ERAP1, IL-23R, IL1R2, ANTXR2, TNFSF15, TNFR1 and TRADD (Australo-Anglo-American Spondyloarthritis consortium [TSAC]; Reveille et al., 2010; Wellcome Trust Case Control Consortium [TASC]; Burton et al., 2010). Many of these candidate genes are hot topics for research recently (Campbell et al., 2011; Chen et al., 2011; Layh-Schmitt & Colbert, 2008; Brown, 2010).

According to the above finding, theories of the disease pathogenesis were proposed. Hypotheses including molecular mimicry, arthritogenic peptide, free heavy chain and unfolded protein response were considered main streams of hypotheses.

3.1 Theory of molecular mimicry and arthritogenic peptides

A striking finding of the similarity of 6 consecutive amino acids of HLA-B27 to 6 consecutive amino acids of nitrogenase from Klebsiella pneumoniae made researchers to propose that after the microbial infection, our immune system mis-recognized self-antigens as a target and launched an autoimmune response (Schwimmbeck & Oldstone, 1998). A Finnish group also identified that two bacterial proteins shared homology with HLA-B27, namely YadA (Yersinia adhesin) and OmpH, outer surface proteins of Yersinia and Salmonella, respectively (Lahesmaa et al., 1991). Further support of this hypothesis is the finding that HLA class II antigen associated with rheumatoid arthritis shared the same amino acid sequences with some viral antigens (Albani & Carson, 1996). Data from serologic studies also indicated that patients with AS had high incidence of antibodies against microbial pathogens (Ewing et al., 1990). This study indicate that AS patient sera contain antibodies which were reactive to K. pneumoniae nitrogenase peptides and HLA B27.1 peptides, and that there are at least two epitopes in the alpha 1 domain at the groove region, that are autoantigenic. However, not all reports are consistent, later reports did not support the finding of antibodies against self-antigens in patients with AS (de Vries et al., 1992).

As mentioned before, the unique amino acid composition, especially in the peptide-binding groove, made B27 molecule distinct. The presence of B pocket fits only arginine inside. This finding together with the knowledge of highly polymorphism of B27 alleles which were differentially associated with AS pave a new way to search for the presence of arthritogenic peptides with the ability to provoke arthritis. All these alleles differed from each other by only one or few amino acid changes, but interestingly, the association with AS were quite different. Most HLA-B27 alleles are found at a very low frequency, their association with AS are largely unknown. B*27:05 is an ancestral type and associated with AS in almost every population in
the world. B*27:04 is very prevalent in Asian country and also is thought to make individual susceptible to AS. On the contrary, B*27:03, B*27:06 and B*27:09 were considered to play a protective role. Rare incidence of these allele-carriers developed AS. B*27:03 was initially thought not to be associated with AS and only was prevalent in Black African population where B*27:05 is also not associated with AS (Hill et al., 1991). Certainly, other genes might be involved in the pathogenesis. Recently, AS patients possessing B*27:03 were found (Reveille et al., 2000). B*27:09 were found in healthy inhabitants of Sardinian island but not in patients, who only carry B*27:05. Although a case of AS possess B*27:09 together with B*14:03, another AS-associated allele in black Africans reported (Cauli et al., 2007). The amino acid sequence of B*27:09 differed from that of B*27:05 only in residue 116 (His vs Asp) (de Castro, 2009). Self-peptide pVIPR binds to B*27:05 in dual conformation but only one conventional form can be bound to B*27:09 (Hulsmeyer M et al., 2004). This different binding link might provoke different T cell response. B*27:06 is found mainly in Southeastern Asia among healthy control, while other B27 alleles were associated with AS. B*27:03 differs from B*27:05 by the Y59H change located in the A pocket. B*27:04 and B*27:06 are closely related differ only by two amino acid changes, namely H114D and D116Y. Different amino acid sequences in the binding groove will change the polar-nonpolar interaction of heavy chain with peptides, hence causes different peptides anchoring to the groove. It was postulated that some disease-causing alleles of HLA-B27 selectively bound arthritogenic peptides derived from several intracellular parasites which were claimed to be triggering agents in reactive arthritis. Even more, some investigators found that peptides derived from self antigen, including peptide from HLA-B27 itself and cartilage were found to trigger CD8+ T cell response (Kuhne et al., 2009; Atagunduz et al., 2005). Three self peptides derived from cartilage/bone proteins showed homology to sequence of protein from arthritogenic bacteria. One of them, peptide PRGLLAWISR derived from chondroitin sulfate N-acetylgalatosaminyltransferase 1 shared 8 amino acids with FhuB protein from Yersinia Enterocolitica and 7 amino acids with intracellular attenuator protein A from Salmonella Typhimurium (Dror LB et al., 2010). The presence of self peptide in the HLA binding groove with homologous sequence from arthritogenic bacteria forms the cornerstone of molecular mimicry. It is interesting to note that in addition to HLA-B27, HLA-B39 had similar B pocket was found to be associated with ankylosing spondylitis in those who were HLA-B27 negative. It was considered to harbor same peptide repertoire with HLA-B27 (Yamaguchi et al, 1995). Another observation is that HLA-B*14:03 is a major MHC molecule associated with AS in Africa, it differs from HLA-B*27:05 at 18 positions and shares only 3-5% peptide repertoires (Lopez-Larrea et al., 2002). Two important papers showed that CD8+ cytotoxic T cells are not essential for the arthritis to develop. In these observations, May et al use monoclonal antibody to deplete CD8+ T cells from peripheral circulation, however, arthritis and colitis still develop in the HLA-B27 transgenic rat (May et al.,2003). In addition, the same conclusion was obtained by the chemical deletion of CD8a gene expression which eliminated CD8+ T cells from peripheral blood (Taurog et al, 2009). The other observation revealed that CD4+ T cells, when transferred to athymic nude rat which had high level of HLA-B27/hβ2m expression in the bone marrow, developed arthritis (Taurog et al., 1999).

3.2 Free heavy chain theory

Another models focus on the molecular stability of tri-molecular complex indicating that due to unique amino acid composition at interface between HLA-B27 and beta2-
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Microglobulin, the tri-molecular complex is not stable enough. Only HLA-B27 was found to be able to express as free form. Amino acid residue 9 in the floor of β pleated sheet is critical for the stability of the heavy chain/β2-microglobulin complex. All the HLA-B27 subtypes contain histidine at this site, interestingly, two other class I molecules, HLA-B73 and HLA-B40, which had been reported in a few cases of spondyloarthropathies, were found to have histidine at amino acid residue 9 (David, 1997). Histidine at this position was claimed to weaken the non-covalent interaction between heavy chain and β2-microglobulin. The unstable structure made HLA-B27 dissipate from beta2-microglobulin and presented as free form on the cell surface. It was proposed that free HLA-B27 bound different peptides from those stable forms. Higher percentage of free heavy chain-carrying monocytes was found in the peripheral blood and synovial fluid in patients with AS compared to normal population. The level of free heavy is correlated with sedimentation rate (Tsai et al., 2002). In addition, as mentioned before, in the presence of unpaired Cys67 free heavy chains have been shown to form homodimer. Expression of heavy chain homodimer on the surface of cell lines and AS patients' peripheral blood mononuclear cells was observed. (Kollnberger et al., 2002)

This HC homodimer was found to bind to NK inhibitory receptors KIR3DL1 and KIR3DL2 and LILRA1, LILRB2 alleles on the surface of NK, T and B cells. Patients with ankylosing spondylitis have higher level of Th17 cells expressing KIR3DL2 and responsive to B27 HC homodimer (Bowness et al., 2011). This hypothesis postulates that through this interaction, NK cells, B cells and T cells were activated to induce the inflammatory reaction.

3.3 Unfolded protein response theory

In the last decade, another new theory was proposed calling "unfolded protein response". In this theory, researcher proposed that due to its unique structure, i.e.; the presence of B pocket and free form, HLA-B27 molecule is not properly folded in the endoplasmic reticulum. The accumulation of unfolded proteins in the endoplasmic reticulum induced stress reaction in the organelle and hence triggered inflammatory response. Most evidences came from animal study. Khare et al found high incidence of joint inflammation and ankylosis when HLA-B27 was transgenic into β2-microglobulin deficient mice (Khare et al., 1995). Later, investigator found a deficiency in class I molecule expression either due lack of peptides (TAP/TAP) or β2-microglobulin was able to induce spontaneous inflammatory joint disease (Kinsbury et al, 2000). In animal study, transgenic rats were found to have increased IL-23 secretion when unfolded protein response was triggered by B27 molecule in the presence of pattern recognition receptor agonist (Colbert et al., 2010). This finding reminds us that IL-23R was found to be a susceptibility gene from genome-wide scan. Another observation from genome-wide scan shows that one of the peptide-trimming peptidase: ERAP1 is associated with AS. Defect in the function of ERAP1 might delay the folding process of HLA class I molecules (Evans et al., 2011). Patients with AS were found to have high level of chaperon proteins which were related to the folding process of class I molecules in their macrophage derived from peripheral joint (Dong et al., 2002).

4. Conclusion

In conclusion, the high association of HLA-B27 with ankylosing spondylitis paved the path to the resolution of pathogenesis of this disease. Identification of this important finding
might open ways to design new treatment modalities and prevent the occurrence of the disease. Evidence from both epidemiology and transgenic animal studies further widen our vision. Although none of the above theories can explain all the phenomena we observed before, newer data from genome-wide scan can further supplement the missing link.

5. References


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The first section of the book entitled Clinical and Molecular Advances in Ankylosing Spondylitis is a review of the clinical manifestations of Ankylosing Spondylitis (AS) and Spondyloarthritis (SpA). The book includes chapters on Bone Mineral Density measurements, two chapters on the temporomandibular joints, axial fractures, clinical manifestations, diagnosis, and treatment. Molecular genetics and immune response are analyzed in the second section of the book; information on HLA-B*27, other MHC genes and the immune response of AS patients to bacteria is reviewed and updated. Two chapters are dedicated to recent information on non-MHC genes in AS susceptibility, and to new data on disease pathways generated from gene expression studies on peripheral blood.

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