Immunopathogenesis of ankylosing spondylitis

Ankylosing spondylitis is a model immunogenetic disease with major common and rare genetic risk factors, likely environmental contributors to its pathogenesis and, to date, no treatment that has been shown to induce disease remission in long-term studies. The discovery of the association of HLA-B27 with the disease in the early 1970s triggered extensive efforts to elucidate the mechanism of this association. However, the precise role of HLA-B27 in ankylosing spondylitis pathogenesis remains unclear. In recent years, rapid progress made in the discovery of non-MHC genes involved in susceptibility to ankylosing spondylitis has combined with increasing ability to investigate the immune system to make rapid progress in unraveling the etiopathogenesis of the condition.

KEYWORDS: ankylosing spondylitis ■ antigen processing ■ HLA-B27 ■ inflammation ■ innate immunity ■ T cell

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How does HLA-B27 cause ankylosing spondylitis?

The major models that have been proposed can be divided into those that relate to the canonical function of HLA class I alleles in peptide presentation to the immune system, and those that involve noncanonical properties of HLA-B27 such as its slow folding rate in the endoplasmic reticulum (ER) or propensity to form homodimers (Figure 1).

The major canonical function of HLA class I molecules is the presentation of antigenic peptides to CD8 T lymphocytes. The arthritogenic peptide hypothesis proposes that the native HLA-B27–peptide complexes derived from self-tissues and formed in the ER of ankylosing spondylitis (AS) patients mimic pathogenic microbial structures. These MHC–self-peptide complexes are, therefore, recognized by the immune system as harmful, inducing recruitment of inflammatory T cells [1]. It has been argued by other authors that this model may not be valid because the arthritis in B27 transgenic rats is independent of CD8 lymphocytes [2,3]. However, a caveat to the B27 transgenic rat is that this animal model expresses multiple copies of HLA-B27 and β2-microglobulin per cell, in contrast to humans who have at most two copies of each of these genes. In support of the arthritogenic peptide theory, T cells reactive to self-peptides have been isolated from AS patients [4], although convincing replicated evidence of an AS-specific arthritogenic peptide identified independently by different groups has yet to be reported [5,6].

Class I molecules such as HLA-B27 are composed of three 'subunits': a polymorphic heavy chain that noncovalently associates with the monomorphic β2-microglobulin and finally a short peptide. Assembly, or folding, of this complex occurs in the ER in a highly ordered manner [7]. Compared with other HLA molecules, HLA-B27 is slow to fold and associate with β2-microglobulin [8]. The unfolded protein response (UPR) hypothesis postulates that misfolding of HLA-B27 triggers an intracellular signaling cascade in the ER that, in macrophages, stimulates production and secretion of IL-23. Evidence in support of the UPR hypothesis is strongest in the B27-transgenic rat model [9], but has also been reported in the synovium of AS patients [10].

Once on the cell surface, β2-microglobulin can dissociate from the heavy chain, leaving a free heavy chain. These heavy chains can then form homodimers. Heavy chain homodimers can be recognized by some natural killer (NK) cells and T cells. Recognition of heavy chain homodimers is allele-specific and in the case of HLA-B27 homodimers a recent report has shown that KIR3DL2+ CD4 T cells recognize these structures and secrete IL-17 in response [11].

Of the >90 subtypes of HLA-B27 that have been reported to date, most are too rare to determine their relative strength of association with AS. However, it is clear that the Asian subtype *HLA-B*2706* and Sardinian subtype *HLA-B*2709* have reduced association with AS compared with the subtypes *HLA-B*2702*, **2703*, **2704*, **2705* and *B*2707*. Any theory as to the mechanism by which HLA-B27 induces

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AS needs to also explain how the different subtypes have different strengths of association with disease. In the author's view the relationship between the different models and B27-subtype variation has not yet been fully established, and as such will not be discussed further here.

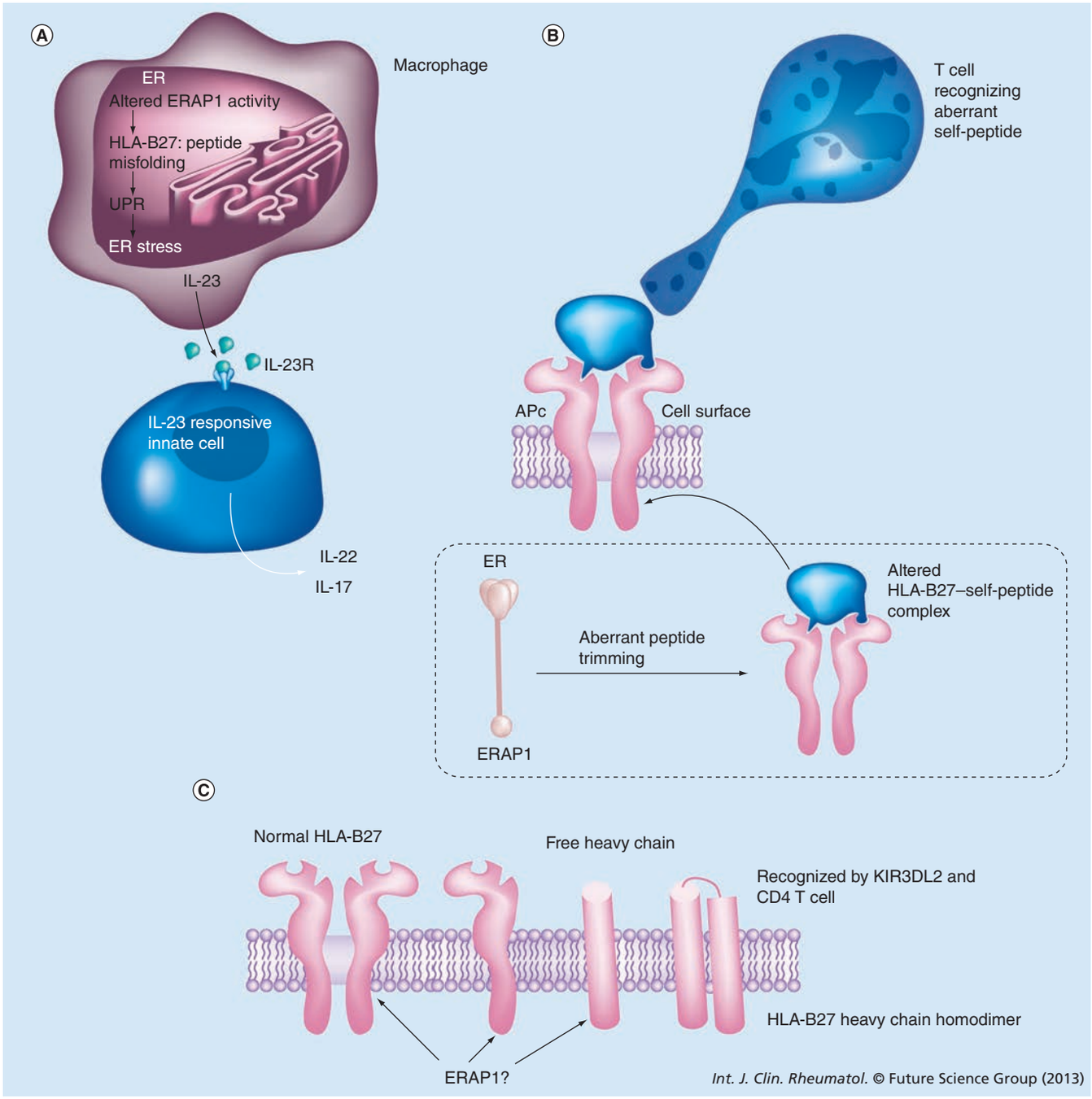

Figure 1. Multiple roles for HLA-B27 in the pathogenesis of ankylosing spondylitis. A number of models have been proposed for how HLA-B27 contributes to ankylosing spondylitis pathogenesis. **(A)** Studies in the HLA-B27 transgenic rat model of ankylosing spondylitis have demonstrated that misfolding of HLA-B27 in the ER induces a cascade of intracellular events collectively termed the UPR. Induction of the UPR triggers secretion of pathogenic IL-23 from macrophages. **(B)** The arthritogenic peptide theory suggests that in ankylosing spondylitis patients the native HLA-B27–self-peptide complexes mimic microbial peptide structures and are recognised by the immune system as harmful, triggering an inflammatory reaction. **(C)** Residues in the binding pocket of HLA-B27 are capable of forming disulfide bonds with other HLA-B27 molecules allowing homodimer formation. B27 homodimers are subsequently recognized by immune cells triggering IL-17 secretion. Experimental support exists for each of these models. Determination of the hierarchy of these models remains an important challenge for future research.
APc: Antigen presenting cell; ER: Endoplasmic reticulum; UPR: Unfolded protein response.

ERAP1 & AS

ERAP1 was one of the first non-MHC genetic associations reported with AS [12]. This association has been widely replicated in white European and East Asian populations. At least two separate AS-associated haplotypes have been identified, tagged by the nonsynonymous SNP rs30187 (which genetic evidence suggests is directly disease-associated) and rs10050860 [13]. These SNPs interact with HLA-B27, such that they only show association with AS in HLA-B27 positive cases [13], representing the first confirmed example of gene–gene interaction in any common human disease. *ERAP1* is also associated with psoriasis, and interaction has now also been reported between the main psoriasis-associated HLA class I allele, HLA-Cw6 and *ERAP1*, with the *ERAP1* association being observed in HLA-Cw6 positive cases [14]. This suggests that HLA-Cw6 is likely to operate to cause psoriasis by similar mechanisms to those by which HLA-B27 induces AS.

It remains unclear how ERAP1 contributes to the pathogenesis of AS. Two main functions of ERAP1 have been reported. First, ERAP1 trims peptides that have been processed through the proteasome from 13–15 amino acids in length down to eight or nine amino acids, which is the ideal length for presentation on MHC class I [15]. As such, ERAP1 is considered to be a 'molecular ruler'. Second, *in vitro* studies have reported that ERAP1 acts as a cytokine 'sheddase', cleaving cytokine receptors off the cell surface, including IL-6R, IL-1R2 and TNF-R [16–18], thereby altering the ability of those cytokines to signal. However, we have demonstrated that spleen cells from ERAP$^{-/-}$ mice did not show altered cleavage of IL-6R and TNF-R *in vitro* [13]. Together, these data point towards a primary role for ERAP1 in AS to be that of a molecular ruler rather than altering cytokine signaling.

The x-ray crystal structure of ERAP1 reveals that the primary AS-associated polymorphisms are found at the hinge region of the protein, which controls enzyme activity by determining whether ERAP1 is in a closed (active) or open (inactive) confirmation [19]. In AS, the protective variant of the *ERAP1* SNP rs30187 causes a significant reduction in enzyme activity [13]. The protective variant is also associated with decreased HLA-B27 molecular stability, whereas an AS-associated variant caused efficient peptide trimming and high HLA-B27 stability [20].

ERAP1 polymorphisms could play a part in all three models for how HLA-B27 functions in AS. Altered rates of peptide trimming caused by AS-associated *ERAP1* polymorphisms probably lead to cell surface expression of aberrant MHC–peptide complexes, which subsequently elucidate an inflammatory immune response. Alternatively, altered enzyme activity may affect the rate at which HLA-B27–peptide complexes fold in the ER, which may, in turn, alter the levels of UPR-derived inflammatory cytokines secreted by macrophages. Finally, *ERAP1* variants have recently been reported to alter levels of cell-surface free heavy chain in a HLA-B27 allele-specific manner, although whether these findings were influenced by the differences in rates of folding of HLA-B27 subtypes is unclear [21]. Whatever the mechanism(s) of action of ERAP1 in AS, this enzyme certainly warrants further investigation and may provide a therapeutic target for AS, especially since *ERAP1*$^{-/-}$ mice show no phenotype other than increased susceptibility to toxoplasma infection [22]. *ERAP1* variants have also been reported to be associated with congenital toxoplasmosis [23].

IL-23 signaling

IL-23 is a key cytokine in the development of IL-17- and IL-22-secreting cells. IL-23 signals through a receptor consisting of the specific IL-23 receptor (IL-23R) subunit and IL-12Rβ1, also shared with IL-12R [24]. Polymorphisms in IL-23R are associated with AS [12], psoriasis [25] and inflammatory bowel disease (IBD) [26]. Under physiological conditions IL-23-, IL-17- and IL-22-producing cells are enriched in gut mucosa and play important roles in regulating intestinal health. Loss of IL-23 signaling renders mice resistant to a number of autoimmune diseases, including collagen-induced arthritis [27] and experimental autoimmune uveitis [28].

While it was initially thought that CD4 T cells ('T_H17 lymphocytes') were the most important cell type involved in IL-17 responses, recent research has identified additional cell types to be critical to IL-17-mediated inflammation. Generation of the IL-23R-GFP reporter mouse was critical to much of the recent appreciation of the diversity of IL-23 responsive cells [29]. In contrast to the dogma at the time, the IL-23R-GFP mouse showed that only approximately 1.2% of cells of IL-23R+ cells in lymphoid tissues were CD4 T cells. Approximately 40% of IL-23R+ cells expressed a γδ T cell receptor, while macrophages and dendritic cells were other prominent IL-23 responsive cells. In the lamina propria of these mice, γδ T cells again were the main IL-23R+ population (~65%) and lymphoid tissue inducer-like

(LTi-like) cells accounted for much of the remaining population of IL-23 responsive cells [29]. This seminal research sparked great interest in the diversity of IL-17 secreting cells in autoimmunity and has led to the recent description of noncanonical sources of IL-17 in animal models and human patients.

Noncanonical sources of IL-17

γδ T cells

γδ T cells account for approximately 1–5% of circulating T cells in healthy individuals, but are prominent at epithelial surfaces such as the gut and skin, where they can account for up to 50% of T cells. Few γδ T cells express either CD4 or CD8 coreceptor molecules. Therefore, they are considered to be able to recognize antigens directly without a need for costimulation, and thus have the capacity to respond very rapidly to antigenic challenge [30,31]. γδ T cells not only bear an antigen-specific T-cell receptor, but also have many properties of cells of the innate immune system, including expression of the major innate immunity receptors, Toll-like receptors. γδ T cells also express dectin-1, which recognizes microbial β-glucans, including curdlan. Expression of these receptors supports a role for γδ T cells in early responses to microbes. Of further relevance, we and others have recently confirmed that *CARD9*, part of the dectin-1 response pathway, is a susceptibility gene for AS as well as for IBD [32,33].

γδ T cells are potent producers of inflammatory cytokines such as IFN-γ, TNF-α and IL-17 [34,35]. In response to *Escherichia coli* infection, IL-17 secreting γδ T cells are critical for recruitment of neutrophils, and antibody depletion of γδ T cells reduces both IL-17 secretion and neutrophil infiltration to the site of infection [35]. Within the context of inflammatory diseases, γδ T cells are pathogenic in the experimental autoimmune encephalomyelitis mouse model of multiple sclerosis [36], the collagen-induced arthritis model [37] and mouse models of colitis [38], and IL-17-secreting γδ T cells are expanded in patients with AS [39]. IL-17-producing γδ T cells induce disease in experimental autoimmune encephalomyelitis mice and amplify IL-17 production by T_H17 cells [40] and recruitment of IL-17-secreting neutrophils [41]. Furthermore, γδ T cells enhance inflammation by restraining the effect of regulatory T cells through an IL-23-dependent mechanism [42]. Intraepithelial γδ T cells also play an important role in modulating intestinal epithelial growth through secretion of FGF [43]. Alterations to γδ T-cell numbers or functions may, therefore, have profound effects on intestinal health.

KIR3DL2+ T cells

Killer-cell immunoglobulin-like receptors (KIRs) are a family of MHC class I-binding receptors expressed on the surface of NK cells and subsets of T cells. KIR3DL2 expresses three immunoglobulin-like domains that normally recognize HLA-A3 and A11 [44]. However, Bowness' group have shown that KIR3DL2 recognizes HLA-B27 homodimers, but not HLA-B27/β2-microglobulin/peptide complexes. Furthermore, they have shown that CD4 T cells expressing KIR3DL2 secrete large amounts of IL-17 upon recognition of HLA-B27 homodimers. They have further demonstrated that KIR3DL2+ CD4 T cells are enriched in the circulation of AS patients and that these cells account for the majority of IL-17-secreting CD4 cells in the circulation of AS patients [11].

NKT cells

Similar to γδ T cells, NKT cells are found in much larger numbers at epithelial surfaces than elsewhere in the body. NKT cells are characterized by expression of an invariant T cell receptor, Vα24Jα18 in humans and the orthologous Vα14Jα18 in mice. NKT cells recognize glycolipid structures presented to them by the nonclassical antigen-presenting molecule CD1d. Similar to γδ T cells, NKT cells are rapid responders to antigenic stimuli and are capable of producing a range of immunoregulatory cytokines [45–48]. NKT cells have protective roles in models of arthritis [49] and spondyloarthropathy (SpA) [50].

Mast cells & neutrophils

Mast cells and neutrophils have recently been described as major sources of IL-17 in inflamed joints in SpA [51,52]. Appel and coworkers examined facet joints of AS patients and OA patients, and showed that innate immune cells, rather than CD4+ T cells, are the major source of IL-17 at inflamed facet joints in AS patients with advanced disease. Using immunohistochemistry they identified $CD15^+$ neutrophils and myeloperoxidase+ myeloid cells as the major source of IL-17 in axial SpA [51]. Noordenbos and coworkers have shown that mast cells are increased in the synovium of SpA patients compared with rheumatoid arthritis controls, and that mast cells expressed more IL-17 in SpA than rheumatoid arthritis synovitis [52]. IL-17 production was observed from neutrophils and mast cells, but not $CD3^+$ lymphocytes, and mast cells were the

major IL-17-expressing cell in SpA synovium. However, it remains unclear from these studies whether mast cells in inflamed joints are actually secreting IL-17 or simply sequestering IL-17 produced by other cell types. It is also unknown what recruits mast cells to the joint. Are these in fact regulatory mast cells that become reprogrammed at the joint by an inflammatory microenvironment to which they subsequently contribute proinflammatory cytokines? Since mast cells are rarely found in the circulation and they are troublesome to functionally assess once isolated from tissues, functional analysis of mast cells has proven difficult. However, it is interesting to speculate what effect mast cell depletion would have on the course of disease in AS patients.

CD4-CD8- T cells

Interest in the role of CD4-CD8- cells in AS has been driven recently by the work of Cua and colleagues [53]. Using minicircle DNA technology to overexpress IL-23 they demonstrated that IL-23 alone is sufficient to induce enthesitis. Entheseal inflammation in their model is mediated by CD4-CD8- cells, which also express IL-23R and RORγt (the transcription factor necessary for synthesis of IL-17 and IL-22). Entheseal inflammation in this model was independent of canonical T_H17 cells. They show bone remodeling at entheses similar to that seen in AS. IL-17 and IL-22 secreted from the CD4-CD8- T cells are important for disease progression in this model, but IL-22 seems to be particularly important for bone remodeling. Interestingly, these CD4-CD8- T cells expressed promyelocytic leukemia zinc finger, which facilitates rapid cytokine secretion after cellular activation. These data suggest that these cells may be an innate-like cell type. They did not, however, express KIRs, and are therefore not likely to be homologues of KIR+ T cells described in humans [53]. As such, they are unlikely to be a 'unifying' cell type that would allow integration of genetic polymorphisms in HLA-B27 and IL-23R. This study still raises questions about the source of IL-23 that initially drives IL-17 and IL-22-mediated inflammation in AS. It is interesting to speculate a role for the gut here.

The role of the gut in AS

The relationship between gut and joint inflammation in SpA has a strong underlying genetic component. The strong cofamiliality of AS and IBD provides indirect evidence for the existence of shared genetic risk factors between the two diseases. A study of Icelandic families showed that first- and second-degree relatives of patients with AS have 3.0- and 2.1-times higher risk of IBD than the general population [54]. As the number of genes known to be involved in these two diseases has increased, the shared genetic risk factors between the two have become apparent. In 2010, Danoy *et al.* studied genes known to be associated with IBD in a large AS cohort [55]. New loci and genes were identified, and of particular note were genes involved in the IL-23 pathway, such as *STAT3, IL23R* and *IL12B* [55]. It is likely that as more genes are discovered to be associated with AS, the overlap of genetic factors involved with gut and joint inflammation will increase.

The 'unusual' nature of gut resident immune cells is widely known and accepted. The nature of these cells and their position as sentinels at a gateway between the external and internal world has been reported and discussed at great length in the scientific press. From the point of view of AS it is intriguing to note that many cell types that are important in mucosal immunology are also linked with roles in AS, including γδ T cells, NKT cells and CD4-CD8- T cells. IL-23 is produced by the gut and is active at mucosal surfaces [56] and IL-23 levels are elevated in the terminal ileum of AS patients [57]. What triggers elevated secretion of IL-23 AS terminal ileum remains unknown, but the role of the gut microbiome and modification of epithelial tight junctions in the AS gut are topics of hot interest currently (Figure 2). A potential hypothesis for the etiopathogenesis of AS is that it is caused by excess IL-23 and downstream cytokine production due to chronic effects of the gut microbiome. This would be consistent with *in vitro* studies suggesting that HLA-B27 is associated with a reduced ability to kill certain bacteria. Thus HLA-B27 may operate in AS by effects on the gut microbiome, perhaps leading to microbial dysbiosis or increased microbial invasion across the intestinal mucosa, in turn driving IL-23 production. Further studies, particularly in humans and in animal models of disease, are ongoing to research this theory.

An important question now is do circulating gut-derived noncanonical IL-23R+ cells respond directly to IL-23 signals in the intestine and spread inflammation systemically in genetically susceptible AS patients? NKT-cell studies give us some insight into how the gut may be important in AS. Recently, it has been shown that microbial stimulation of NKT cells in the gut of mice affects NKT cell phenotypic and functional

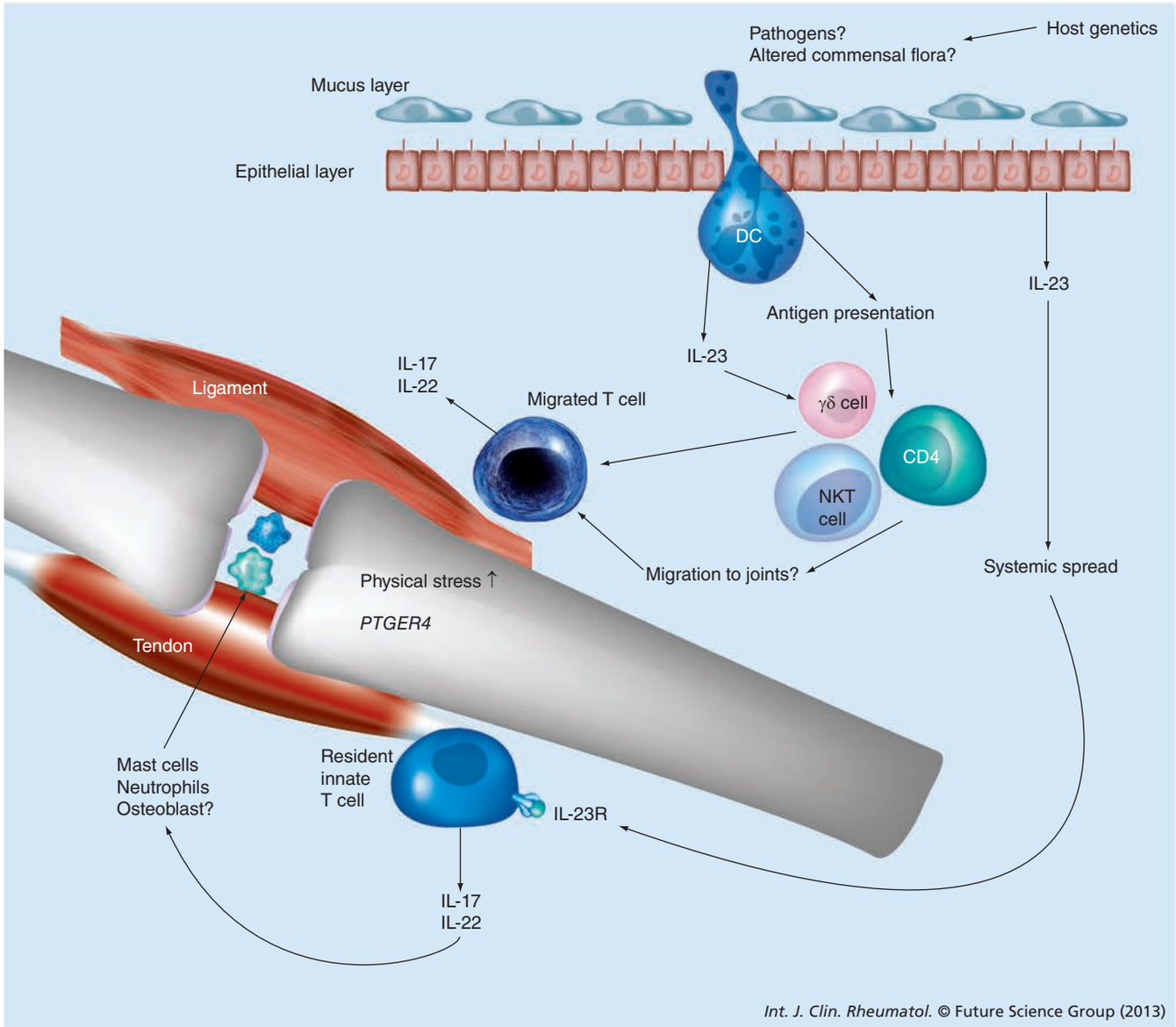

Figure 2. The gut and noncanonical sources of IL-17 probably play central roles in ankylosing spondylitis pathogenesis. Our understanding of the role of IL-23 signaling in ankylosing spondylitis pathogenesis is rapidly changing. Noncanonical sources of IL-17 have been described recently both in the circulation and in inflamed tissues. KIR3DL2+ CD4 T cells secrete IL-17 upon recognition of HLA-B27 homodimers on the surface of macrophages. Circulating γδ T cells in ankylosing spondylitis patients express elevated levels of IL-23R and secrete large amounts of IL-17, although the antigenic drive for this is unclear. In the gut, alterations to the commensal microflora or invasion by pathogenic bacteria may alter the structure and function of the epithelial barrier, signaling inflammatory responses mediated by CD4, γδ and NKT cells (and perhaps other cells not yet described) in an IL-23-dependent manner. In joints, inflammatory mast cells and neutrophils may contribute to inflammation through IL-17-dependent mechanisms. At entheses, CD4-CD8- T cells can respond to IL-23 signals alone and secrete IL-17. Many unknowns remain, including antigenic triggers, contribution of genes regulating mechanical stress (such as *PTGER4*), relative roles for IL-17 and IL-22, and the identity of pathogenic versus 'bystander' inflammatory cells.
DC: Dendritic cell; NKT: Natural killer T.

maturation [58]. Given that NKT cells have protective roles in models of arthritis [49] and SpA [50] their functional maturation in the gut provides evidence for a role for mucosal T-cell priming in inflammatory joint disease.

A key role for IL-23/IL-17 in pathogenesis of AS is supported by genetic and clinical trial data. The recent descriptions of various populations of IL-17-producing cells in AS, including KIR3DL2+ CD4 T cells, γδ T cells, neutrophils and mast cells, raises many questions about the role of each of these populations of IL-17+ cells in AS. How are each of these cell types activated to produce IL-17? We know already, for example, that KIR3DL2+ CD4 T cells recognize HLA-B27 homodimers, but nothing is known about

activation of other noncanonical sources of IL-17. Do these cells produce other IL-23-dependent cytokines (e.g., IL-22 or other IL-17 isotypes) that may affect their function? Which, among these cell types, are pathogenic and which are mere 'bystanders' in the inflammatory cascade? Improved understanding of the basic biology of the noncanonical sources of IL-17 is also much needed. We know, for example, that entheseal resident CD4-CD8- T cells can respond robustly to IL-23 signals alone and that they exist in a preactivated state, but little is known of the functional response characteristics of other IL-17-secreting innate cells. Together, this information may provide opportunities to target a specific subset of inflammatory cells rather than an entire inflammatory pathway.

TNF-mediated inflammation

TNF antagonists are highly effective in reducing systemic levels of inflammation in AS. The most recent genome-wide association study (GWAS) data identified three genes involved in TNF signaling to be associated with AS: *LTBR, TNFRSF1A* and *TBKBP1*, a component of the TNF receptor signaling pathway [13,59]. Furthermore, TNF overexpression induces a spondyloarthritis-like phenotype, associated with IBD [60]. These findings suggest a central involvement of TNF excess in AS.

Fungal response genes

CARD9 and *PTGER4* were identified by the TASC/WTCCC2 study [13]. CARD9 mediates signals from the innate immune receptors dectin-1 and -2, which recognise β-glucan, a component of fungal and some bacterial cell walls. Signaling through CARD9 induces production of PGE2, the ligand for PTGER4 (prostaglandin E receptor 4, EP4 subtype). β-glucan stimulates IL-17 production in an IL-23-dependent manner and blockade of PGE2 decreases this IL-17 production [61]. More recently, it has been shown that injection of SKG mice with the fungal β-glucan induces development of a SpA-like disease, including Crohn's disease symptoms [62]. Disease development in SKG mice is IL-17 dependent [63]. This model would suggest that SpA could be triggered by pathogens that ligate dectin-1 or -2. PTGER4 also likely plays a role thats links mechanical stress, inflammation and bone formation in AS. PGE2 expression is increased in response to mechanical stress, and has been shown to act through PGE2EP4 to inhibit SOST expression, which would promote bone formation [64]. PGE2 has also been shown to increase IL-23 production by dendritic cells [65], thereby providing a link between inflammation and bone formation in AS.

CD8 T cells

Runx3 is an important transcription factor in the development and differentiation of CD8 T cells. *RUNX3* shows strong association with AS. Furthermore, we have shown that CD8 T-cell counts are reduced in the circulation of AS patients [13]. In addition, healthy controls carrying the AS-associated *RUNX3* polymorphism also display reduced CD8 counts. Suggestive association between *IL7R* polymorphisms and AS has also been reported [13]. IL7R stimulation drives RUNX3 expression [66]. The precise involvement of *RUNX3* and *IL7R* in AS remains unclear, but these associations support the hypothesis that HLA-B27 contributes to AS through a mechanism that involves presentation of antigen(s) to CD8 T cells, after antigen processing by ERAP1.

Current & emerging therapies

TNF antagonists have been used for several years to effectively reduce systemic inflammation in AS and other inflammatory joint diseases. However, TNF inhibition does little to prevent or even slow disease radiographic progression, although effects on aspects such as deformity, disablility, morbidity and mortality remain to be determined. In an era of biological therapies, blockade of IL-1 [67], IL-6 [68], B-cell function [69] and T-cell costimulation [70] have proven at most moderately effective in SpA.

Targeting pathways identified by GWAS is likely to prove highly effective in the treatment of AS. Indeed, the GWAS era has already proven to be therapeutically beneficial in SpA. IL-12/23 blockade is effective in psoriatic arthritis [71] and IL-17 inhibition is effective in the treatment of AS patients with active disease [72]. GWAS findings have also provided the clearest evidence yet that AS is very different in etiopathogenesis to rheumatoid arthritis. Thus, we need therapeutic development pipelines specific for AS, rather than the current model in which pharmaceutical companies trial 'hand-me-down' medications that have proven to be effective in rheumatoid arthritis in particular. Given the marked differences in the genetics of the two conditions, it is not impossible that this practice will end up causing harm, in which medications effective in rheumatoid arthritis actually exacerbate AS. Given the high prevalence of AS and related spondyloarthropathies, there is

clearly a strong healthcare need and commercial argument for more research in treatments specifically for AS.

As we move from GWAS to whole-exome, and eventually whole-genome, sequencing the complete picture of genetic polymorphism in AS will become clearer. The onset of IL-12/23 and IL-17 inhibition strategies in SpA provide effective alternatives to anti-TNF therapy, which although highly effective in most AS patients does not suit all, and does not induce disease remission. These advances are certainly welcomed and while they have proven safe in Phase II clinical trials, blockade of important inflammatory pathways will, to some extent, compromise patient immune responses to infectious agents and transformed cells.

Conclusion & future perspective

Genetic studies have far outpaced functional validation of these genetic findings. The challenge in coming years is to determine true functional drivers of disease, to discern what pathways and/or cell types lie at the top of the pyramid of inflammation in AS so we can shut inflammatory processes off close to their roots. Our understanding of the pathogenesis of AS is likely to explode with the marriage of genetic and functional studies. An era of targeted medicine, where specific cell types or proteins, rather than whole immune pathways, can be investigated is fast approaching. Validation of the genetic signatures of disease in animal models and patient samples is already in full swing for some of the targets identified by GWAS. Whole-exome and whole-genome sequencing will improve the power to determine which pathways require functional validation.

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Executive summary

How does HLA-B27 cause ankylosing spondylitis?

- HLA-B27 may induce ankylosing spondylitis (AS) either by mechanisms involving antigen presentation to the immune system, leading to either autoimmunity or immunodeficiency, or noncanonical mechanisms, such as induction of endoplasmic reticulum stress or aberrant interaction with the immune system of HLA-B27 homodimers.

ERAP1 & AS

- The *ERAP1* association with AS is restricted to HLA-B27-positive cases, and variants associated with protection from AS lead to reduced ERAP1 function.

IL-23 signaling

- The association of variants of *IL23R* and other genes in the IL-23 response pathway highlights this pathway as a key component of the pathogenesis of AS and other related conditions such as inflammatory bowel disease and psoriasis.

Noncanonical sources of IL-17

- A range of different IL-23-responsive cells including γδ T cells, *KIR3DL2*+ T cells, natural killer T cells and CD4-CD8- T cells, as well as mast cells and neutrophils, have been shown to be increased in AS, and may contribute to AS pathogenesis.

The role of the gut in AS

- Genetic evidence strongly suggests that disordered gut immunity is involved in driving inflammation in AS.

TNF-mediated inflammation

- The excellent therapeutic effect of TNF inhibition on inflammation in AS, and genetic association of TNF receptors with the disease, indicates that TNF overexpression is also important in AS inflammation.

Current & emerging therapies

- Genetic findings in AS have pointed to many potential novel therapeutic targets in the disease. Given the high prevalence of AS and related spondyloarthropathies and availability of only one very effective therapy, TNF-inhibition, there is clearly a major need for more research into the development of new therapies for this condition.

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