Pathogenesis of ankylosing spondylitis — recent advances and future directions

Vidya Ranganathan[1], Eric Gracey[1,2], Matthew A. Brown[3], Robert D. Inman[1,2,4,5] and Nigil Haroon[1,4,5]

Abstract | Over the past 5 years, advances in high-throughput techniques and studies involving large cohorts of patients have led to considerable advances in the identification of novel genetic associations and immune pathways involved in ankylosing spondylitis (AS). These discoveries include genes encoding cytokine receptors, transcription factors, signalling molecules and transport proteins. Although progress has been made in understanding the functions and potential pathogenic roles of some of these molecules, much work remains to be done to comprehend their complex interactions and therapeutic potential in AS. In this Review, we outline the current knowledge of AS pathogenesis, including genetic risk associations, HLA-B27-mediated pathology, perturbations in antigen-presentation pathways and the contribution of the type 3 immune response.

[1]*Krembil Research Institute, University Health Network, 1–60 Leonard Avenue, Toronto, Ontario M5T 2S8, Canada.*
[2]*Department of Immunology, University of Toronto, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada.*
[3]*Institute of Health and Biomedical Innovation, Queensland University of Technology, Translational Research Institute, Brisbane, Queensland 4001, Australia.*
[4]*Department of Medicine, Division of Rheumatology, University of Toronto, 399 Bathurst Street, Toronto, Ontario M5T 2S8, Canada.*
[5]*Toronto Western Hospital, 399 Bathurst Street, Toronto, Ontario M5T 2S8, Canada.*

Correspondence to N.H.
Nigil.Haroon@uhn.ca

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Ankylosing spondylitis (AS) is an inflammatory arthritis of the axial skeleton that predominantly affects young men. Although the MHC class I allele *HLA-B*27* confers the greatest genetic risk of AS, genome-wide association studies have revealed more than 60 additional risk factors for the disease. Despite HLA-B27 being linked to AS for more than 40 years, and its role in the presentation of antigenic peptides to T cells being well characterized , the pathogenic role of $CD8^+$ T cells remains to be conclusively demonstrated. By contrast, research over the past 10 years has demonstrated a crucial role for IL-17-producing $CD4^+$ T cells in the pathogenesis of AS. Moreover, subclinical gut inflammation is observed in 60% of patients with AS[1], implicating a potential gut–joint axis in AS pathogenesis. The gut could be the first location of antigenic exposure, followed by activation of pathogenic mechanisms within the joints.

Considerable effort over the past 5 years has been directed towards understanding three major themes, namely intracellular peptide processing and presentation by HLA-B27, the microbiome and its interaction with the immune system, and perturbations of type 3 immunity. Although these processes form the basis of our understanding of AS pathogenesis, a consensus has yet to be reached regarding their mechanistic links with disease[2,3]. In this Review, we discuss advances in our understanding of AS pathogenesis as informed by the 2015 International Genetics of AS (IGAS) congress held in Toronto, Canada as well as the relevant studies published afterwards.

Genetics of AS

Since its discovery in 1973, *HLA-B*27* has undisputedly remained the major genetic risk factor in AS[4]. The polygenic nature of AS has been slowly unravelled over time with the identification of several other MHC genes contributing to the disease risk. Genes in the HLA-B locus commonly confer disease risk, and, importantly, associate with both HLA-B27-positive and HLA-B27-negative cases of AS[5]. However, a major breakthrough in the investigation of AS pathogenesis was the identification of non-MHC genes contributing to AS heritability.

As more genes are being identified, novel molecular pathways that improve our understanding of AS pathogenesis are being discovered. At least 113 genetic variants involved in AS have been identified to date, with 48 of these achieving genome-wide significance[5–9]. Another 65 genes are genome-wide significant for combinations of seronegative diseases (AS, Crohn's disease, primary sclerosing cholangitis, psoriasis and ulcerative colitis) and independently associated with AS itself[9].

A genetic study published in 2007 identified variants among non-HLA proteins as major risk factors for AS, such as those involved in the IL-23 signalling pathway and those belonging to the M1 family of zinc metallopeptidases, such as endoplasmic reticulum aminopeptidase 1 (ERAP1)[6]. Since the discovery of these variants, multiple non-HLA genes have been associated with AS, including *CARD9*, *EOMES*, *IL1R1*, *IL1R2*, *IL6R*, *IL7R*, *IL12B*, *IL27*, *NKX2-1*, *PTGER4*, *RUNX3*, *TBX21*, *TYK2*

Key points

- More than 100 genetic loci have been associated with ankylosing spondylitis (AS), but together they explain less than 30% of AS heritability
- Multiple genes involved in antigen processing and presentation are associated with AS
- Investigation into the cellular sources and regulation of IL-17 production is crucial to understanding the pathogenesis of AS
- Cells involved in type 3 immunity produce IL-17 and include innate lymphoid cells and γδ T cells
- Given the efficacy of the anti-IL-17 monoclonal antibody secukinumab in the treatment of AS, other therapies targeting type 3 immunity could also be effective

and *ZMIZ1* (REF. 10). Interestingly, many of these genes are associated with type 3 immunity, which is mediated by innate and adaptive immune effector cells that commonly express nuclear receptor RORγt and produce cytokines of the IL-17 family (TABLE 1). Analysis of HLA genes has demonstrated that in addition to *HLA-B27*, associations with multiple other HLA risk alleles exist, including *HLA-A*02*, *HLA-B*13*, *HLA-B*40*, *HLA-B*47* and *HLA-DRB1*0103* (REFS 11–13), and *HLA-C*1502* in individuals from East Asia[14], as well as associations with protective alleles such as *HLA-B*7* and *HLA-B*57* (REFS 11–13). The association between HLA-B and AS seems to be explained by the amino acid residue 97 in the HLA-B protein sequence, which lies in the C/F pocket of the peptide-binding groove. *ERAP1* variants are associated with AS only in individuals carrying *HLA-B27* and *HLA-B*40* (REF. 11), indicating that similar mechanisms underlie the function of these alleles. Other HLA alleles interact with *ERAP1* in seronegative diseases (*HLA-Cw*0602* in psoriasis[15], *HLA-B*51* in Behçet disease[16] and *HLA-A29* in birdshot chorioretinopathy[17,18]). These shared associations also explain in part the co-occurrence of different diseases in AS; for example, *HLA-DRB1*0103* is a known risk factor for inflammatory bowel disease (IBD).

The identification in 2016 of a large number of genes associated with AS that have pleiotropic effects in seronegative diseases provided new biological insights that might serve as a basis for hypothesis-driven research[9]. Examples of AS regulators under intense investigation include the IL-23–IL-17 axis as well as DNA (cytosine-5)-methyltransferase 3A (DNMT3A) and DNMT3B, which are key proteins involved in epigenetic regulation. *FUT2*, a gene that determines secretor status (the ability to secrete blood group antigens into bodily fluids), has been shown to determine the microbiome composition in patients with IBD[19], supporting the notion that AS is driven by interactions between the host microbiome and the immune system[20].

Heritability estimates for common genetic variants obtained through the Immunochip platform suggest that AS heritability is higher than that of IBD or psoriasis, but, to date, only approximately 28% of AS heritability has been explained by genetic loci in genome-wide association studies (GWAS)[9]. Clearly, many genetic variants remain unidentified, and the identification of these variants will be valuable given the important biological insights arising from genetic findings. Large-scale studies analysing the whole genome, rather than targeted studies such as those utilizing the Immunochip platform, are warranted, as are studies in unique populations in which new disease-associated variants might be identified[21].

Role of MHC in AS

Among the genes associated with AS described above, *HLA-B27* confers the greatest risk and is present in 85–90% of patients[22]. The predominant physiological function of HLA-B27 is to bind peptides and present them to the surface of nucleated cells for recognition by $CD8^+$ T cells. Furthermore, this polymorphic molecule has a subtype-specific influence on the development of AS[23]. Sequence variations in HLA-B27 subtypes confer differences in peptide-binding ability and protein stability[24].

No definitive 'arthritogenic peptide' triggering an immune response in AS has been identified to date, and qualitative differences between peptides that bind to AS-associated and non-associated HLA-B27 subtypes have not been found[25]. Most peptides binding to HLA-B*2704 and HLA-B*2705 (encoded by AS-associated alleles) can also bind to HLA-B*2706 and HLA-B*2709, which are not associated with AS[25]. In the past 5 years, the focus of research has shifted to quantitative HLA-B27 'peptidome' analyses and, with the advent of data-independent acquisition mass spectrometry, high-quality results have been reported[26]. Peptides that are under-represented on HLA-B*2706 and HLA-B*2709 were quantified with the assumption that a putative arthritogenic peptide would bind preferentially to AS-associated HLA-B27 subtypes than non-AS-associated HLA-B27 subtypes, potentially leading to abnormal immune responses. Interestingly, 26 peptides were presented in lower abundance by non-AS-associated HLA-B27 subtypes, and two peptides, ARYVFQSENTF and ARVLLVPDNTF, showed the lowest abundance. Although most differences in abundance were minimal, the authors claimed that subtle differences in peptide binding between subtypes, which seem to be driven predominantly by variability in the affinity for C and N-terminal ends, could drive pathogenic immune responses[26]. Longer peptides seem to be accommodated by folding the central part of the peptides out of the peptide-binding groove while still maintaining the C and N-terminal anchors. Conformational differences in the same peptide can be observed depending on whether it is bound to HLA-B*2705 or HLA-B*2709 (REF. 27). Advanced, quantitative peptide analyses are required to replicate these findings, especially in the context of T cell activation in patients with AS.

Accumulation of unconventional forms of HLA-B27, such as free heavy chains (FHCs), was recently reported in the gut and synovial tissues of patients with spondyloarthritis (SpA) and *HLA-B27*-transgenic rats[28]. Multiple hypotheses exist regarding the potential pathogenic role of FHCs in AS[29]. Owing to the intrinsic relative instability of HLA-B27 compared with that of other HLA-B alleles, FHC formation is higher with HLA-B27 (REF. 30). FHCs can accumulate in the endoplasmic reticulum (ER),

Genome-wide significance
In most genome-wide association studies, the threshold that an association must reach to be considered statistically significant is a high *P* value ($\geq 5 \times 10^{-8}$) owing to the multiple tests conducted in such studies.

Seronegative diseases
Diseases not associated with serum autoantibodies.

M1 family of zinc metallopeptidases
Aminopeptidases that cleave polypeptides from the N-terminus and are dependent on a single zinc ion for activity.

Immunochip platform
A microarray chip containing probes that recognize approximately 195,000 single nucleotide polymorphisms and 700 small insertion and/or deletions; the main aim of this platform is to fine-map genetic associations identified in 11 autoimmune and inflammatory diseases.

Data-independent acquisition mass spectrometry
Mass spectrometry technique in which all ions generated are fragmented and analysed without pre-selection.

Table 1 | **Global polarization of immunity**

Type of immune response	Cytokine receptor(s)	STAT(s)	Transcription factor	Effector cytokine(s)	Cell types
Type 1	IL-12R	STAT1, STAT4	T-bet	IFNγ	ILC1s, NK cells, cytotoxic $CD8^+$ T cells, $IFN\gamma^+$ $CD4^+$ T cells
Type 2	IL-4R	STAT6	GATA3	IL-4, IL-5, IL-13	ILC2s, $IL\text{-}4^+$ $CD8^+$ T cells, $IL\text{-}4^+$ $CD4^+$ T cells, mast cells, basophils, eosinophils
Type 3	IL-1β, IL-6, IL-23R	STAT3	RORγt	IL-17, IL-22, GM-CSF	ILC3s, MAIT cells, $IL\text{-}17^+$ $CD8^+$ T cells, $IL\text{-}17^+$ $CD4^+$ T cells, neutrophils

Current concepts of innate and adaptive immunity postulate a broad polarization of effector T cell and innate lymphoid cell (ILC) lineages, and involvement of specific myeloid cells. Forkhead box protein P3 (FOXP3) expression and subsequent regulatory activity are thought to be mainly restricted to IL-2-induced and/or TGFβ-induced, STAT5/FOXP3-dependant $CD4^+$ regulatory T cells and, therefore, have not been included in this table. GATA3, trans-acting T-cell-specific transcription factor GATA3; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-4R, interleukin 4 receptor subunit α; IL-12R, interleukin 12 receptor subunit β1; IL-23R, interleukin 23 receptor; MAIT cell, mucosal-associated invariant T cell; NK cell, natural killer cell; RORγt, RAR-related orphan receptor γ; STAT, signal transducer and activator of transcription.

leading to ER stress that in turn triggers the unfolded protein response (UPR). The UPR has been linked to increased production of multiple cytokines, especially IL-23, which is particularly relevant to AS[31]. UPR can be challenging to study in patients with AS owing to tissue-specific responses and the difficulty in obtaining tissue from facet and sacroiliac joints by biopsy. Given that a considerable proportion of patients with AS have gut manifestations, enteric biopsies could be a good tool to study tissue abnormalities in AS. However, a 2013 study showed that autophagy, but not UPR, was activated in gut tissue from patients with AS[32]. Similarly, UPR activation was not observed in the synovium or peripheral blood mononuclear cells (PBMCs) of patients with AS[33]. Autophagy is a cellular process that can remove dying cellular organelles and misfolded proteins to improve cell survival[34]. Autophagy might be directly linked to AS pathogenesis by clearance of misfolded HLA-B27 molecules and regulation of UPR. Changes in autophagy also seem to be tissue-specific, as abnormalities could not be demonstrated in the synovium or PBMCs from patients with AS[35].

An important regulatory process related to ER stress and UPR activation is endoplasm reticulum-associated protein degradation (ERAD). Inhibition of ER degradation-enhancing α-mannosidase-like protein 1 (EDEM1), an ERAD-related molecule, leads to an increase in the number of HLA-B27 dimers[36]. Thus, UPR, autophagy and ERAD are highly interconnected. Whether decoupling these processes results in AS pathogenesis requires further investigation[34].

Extracellular, non-classical HLA-B27 molecules can be pathogenic as they trigger abnormal immune responses. Interestingly, HLA-B27 FHC dimers present on the cell surface of antigen-presenting cells stimulate IL-23 receptor (IL-23R)-positive T cells carrying the killer cell immunoglobulin-like receptor (KIR) 3DL2 to produce IL-17[37]. KIRs can transduce both activating and inhibitory signals; for example, KIR3DL2 is an inhibitory receptor that binds with strong affinity to unconventional, dimeric forms and FHC forms of HLA-B27 and inhibits its inflammatory cytokine production as well as natural killer (NK) cell adhesion[38]. As mentioned above, unconventional HLA-B27 forms are present in the gut and synovium of patients with SpA[28]. Interaction between activated, KIR3DL2-expressing $CD4^+$ T cells and HLA-B27 dimers promotes the expression of T helper 17 (T_H17)-cell-specific transcription factor RORγt and the anti-apoptotic factor B cell lymphoma 2 (BCL-2)[39]. Thus, KIR activation could decrease apoptosis of activated T_H17 cells in AS. Promisingly, in *HLA-B27*-transgenic rats, HD5, a monoclonal antibody that specifically targets HLA-B27 dimers, blocked the interaction between KIRs and HLA-B27 dimers and effectively reduced TNF and IL-17 production by $CD4^+$ T cells[2].

Antigen processing and presentation

Three genes of the M1 family of zinc metallopeptidases have been shown to be associated with AS: *ERAP1*, *ERAP2* and *NPEPPS* (encoding puromycin-sensitive aminopeptidase)[8]. The function of the proteins encoded by these genes is to trim peptides to appropriate lengths for presentation by HLA molecules[40–42]. Protective variants of ERAP1 and ERAP2 have reduced rates of peptide cleavage[5,43] and different functional effects that might lead to increased or reduced availability of antigenic peptides, depending on whether the antigen is created or destroyed by the aminopeptidase. The altered availability of antigenic peptides could have various effects on HLA antigen presentation (FIG. 1), HLA-B27 FHC expression and T_H17 cell activation through the interaction with KIRs[44]. Substrate levels can affect the enzymatic activity of aminopeptidases, and variable effects on aminopeptidase activity as well as FHC expression have been reported in patients with AS[45–47]. Puromycin-sensitive aminopeptidase variants associated with AS have not yet been functionally characterized.

The direct functional effect of ERAP1–HLA-B27 interaction is not clear. Decreased ERAP1 activity was shown to reduce HLA-B27 stability[48]. ERAP1 seems to affect

Autophagy
A process that involves the orderly degradation of dysfunctional intracellular components through their delivery to lysosomes in structures called autophagosomes.

Endoplasmic reticulum-associated degradation
(ERAD). A process that facilitates the degradation of misfolded proteins in the endoplasmic reticulum by transporting them to the cytoplasm, where ubiquitylation followed by proteasome-mediated degradation occurs.

peptide handling by AS-associated HLA-B27 subtypes to a greater extent than it affects HLA-B27 subtypes not associated with AS, which explains the previously reported differences in ERAP1 functional interaction with HLA-B27 subtypes[24,47]. Interestingly, experimental models of influenza infection in HLA transgenic mice have demonstrated that generation of the HLA-B27 immunodominant viral epitope, but not the HLA-B7 viral epitope, is dependent on ERAP1 (REF. 49). Two studies suggested that ERAP1 deficiency leads to increased number of FHC dimers and longer HLA-B27-associated peptides, whereas another study showed a decrease in FHC expression resulting from reduced expression of ERAP1 (REFS 44,47,50). Another study reported that AS-protective ERAP1 variants do not increase ER stress[51]. Some of these discrepancies could be explained by the methodology used in different studies, and extreme reduction of ERAP1 expression in knockdown experiments might not reflect the genetic effects occurring *in vivo*. Furthermore, the use of fresh PBMCs, cryopreserved primary cells or cell lines might lead to different results. Substrate concentrations might vary in different experimental conditions and, accordingly, the trimming efficiency of ERAP1 can change considerably[45].

Familial studies show an association between AS and a specific *ERAP1–ERAP2* haplotype[52]. Interestingly, ERAP1 and ERAP2 can form heterodimers, resulting in enhanced peptide trimming efficiency[53]. However, a human polymorphism resulting in the loss of ERAP2 expression does not alter the expression of HLA-B27 on the cell surface[54], suggesting functional differences between ERAP1 and ERAP2. A 2016 study reported a crucial role for ERAP2 in shaping the HLA-B27-bound peptidome through degradation of N-terminal basic residues and generation of nonameric ligands[55]. Additional studies on the functional relevance of the *ERAP2*–AS association are warranted.

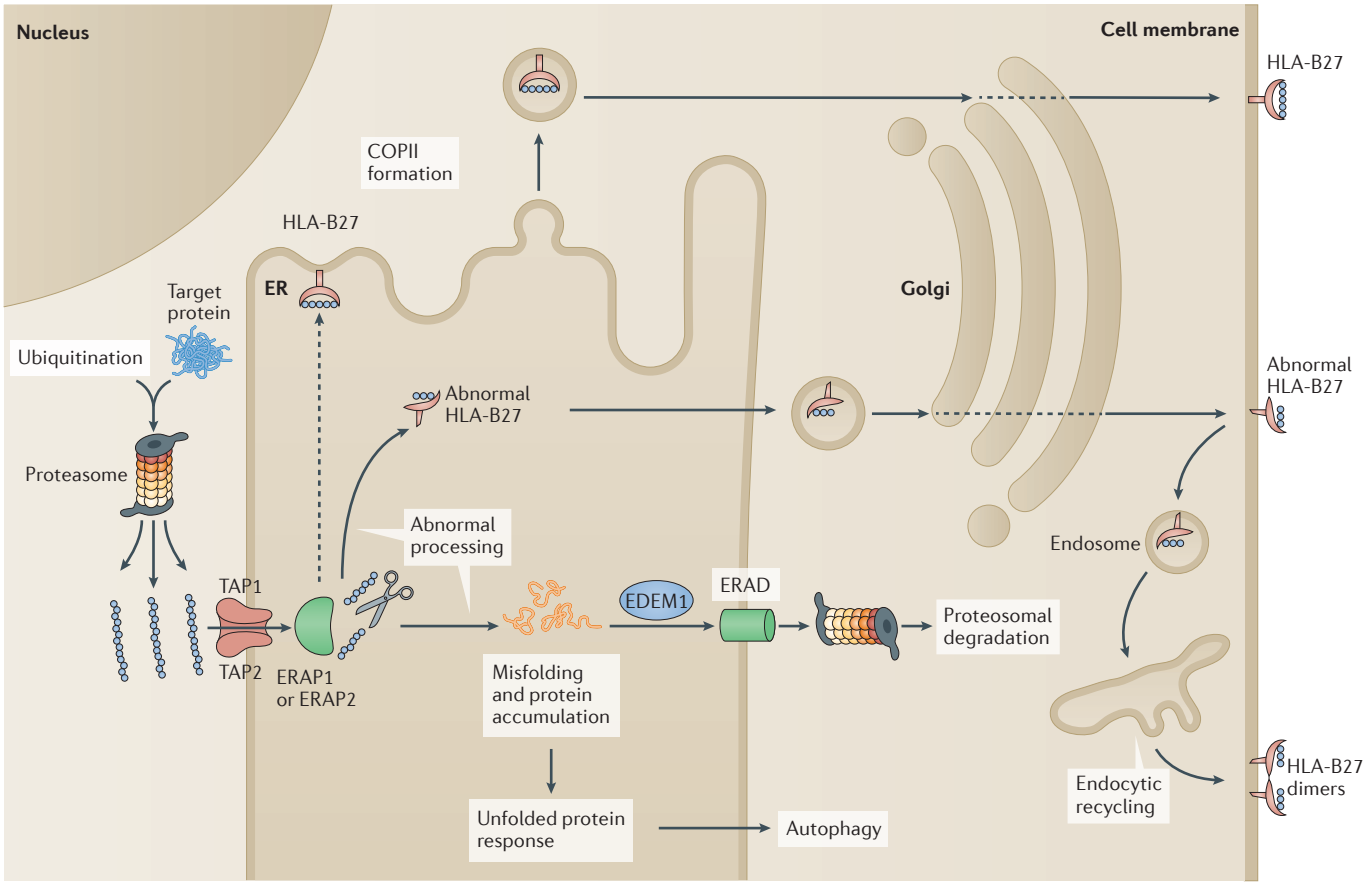

Figure 1 | **Antigen processing and presentation: potential link to AS.** Proteins destined to be degraded are ubiquitylated and broken down into peptides by the proteasome. Peptides that have an excessive number of amino acids at the N-terminal, which cannot be accommodated on class I MHC molecules, enter the endoplasmic reticulum (ER) and are further trimmed by endoplasmic reticulum aminopeptidase 1 (ERAP1) and ERAP2. Peptides of appropriate length are loaded onto class I MHC molecules such as HLA-B27 to form a peptide–HLA-B27–β2m heterotrimer. This complex is then transported to the cell surface via the Golgi apparatus. Like other molecules destined to leave the ER, peptide–HLA-B27–β2m is transported through coat protein complex II (COPII) vesicles. COPII vesicles are formed at a ribosome-free ER-exit site, and protein transport protein SEC16A regulates this process. Abnormal peptide complexes can form as a result of altered ERAP1 or ERAP2 activities, resulting in abnormal surface peptide–HLA-B27–β2m complexes. Unstable peptide–HLA-B27 complexes can accumulate in the ER, triggering the unfolded protein response (UPR), ER-associated protein degradation (ERAD) and autophagy. Defects in COPII vesicle formation, ERAD or autophagy could potentially amplify UPR responses. Furthermore, free heavy chains of misfolded HLA-B27 can combine to form dimers. AS, ankylosing spondylitis; EDEM1, α-mannosidase-like protein 1; TAP, transporter associated with antigen processing.

Coat protein complex II
Vesicle coat protein that aids anterograde transport of proteins from the endoplasmic reticulum to the Golgi apparatus.

SKG mice
Mice carrying a hypomorphic single nucleotide polymorphism in ZAP70 (a T cell receptor signalling molecule), which predisposes T cells to a T helper 17 cell phenotype. Under specific pathogen-free conditions, SKG mice are disease-free; however, a single dose of curdlan, an IL-23-inducing molecule, induces progressive spondyloathropathy characterized by axial and peripheral arthritis, dermatitis and colitis.

Innate-like lymphocytes
Cells of the lymphocyte lineage that express T cell receptors of limited diversity and are restricted by non-classical MHC molecules such as CD1 or MR1. These cells typically recognize non-peptide antigens and are activated faster than regular peptide-restricted adaptive immune cells.

Mucosal-associated invariant T (MAIT) cells
A population of innate-like lymphocytes that recognize bacterially derived vitamin B metabolites presented on the non-classical MHC molecule MR1.

Exome sequencing performed in a multigenerational family comprising several patients with axial SpA identified a rare 9 bp in-frame mutation in exon 3 of *SEC16A*[21], a gene encoding protein transport protein SEC16A, which is involved in the formation of coat protein complex II (COPII) and trafficking of molecules from the ER to Golgi apparatus (FIG. 1). Among nine family members who had genetic deletions in *HLA-B27* and *SEC16A*, seven developed axial SpA. These deletions led to alterations in the secondary structure of the encoded proteins but did not affect their total expression levels. Gene–gene interaction (with potential functional consequences) between *HLA-B27* and *SEC16A* seems to exist in AS as none of the family members who carried the *SEC16A* deletion developed disease in the absence of *HLA-B27* (REF. 21). SEC16A variants could affect trafficking of HLA-B27 to the Golgi apparatus and, subsequently, to the cell surface. This effect can, in turn, affect immune responses as well as ER accumulation of proteins such as HLA-B27. Studying the role of this and other molecules related to familial AS should provide insight into the pathogenesis of AS.

IL-17 and type 3 immunity in AS

The speed at which the role of IL-17 has been recognized in AS is remarkable. Soon after the levels of this cytokine were found to be elevated in serum, synovial fluid, joints and $CD4^+$ (T_H17) cells from patients with AS[56–58], GWAS identified several IL-17-related genes as risk factors for AS development[7,8,9]. The central role of IL-17 in animal models of SpA, such as SKG mice and *HLA-B27* transgenic rats[59,60], supported therapeutic targeting of IL-17 in patients with AS. A study published in 2016 reported an increase of IL-17 levels specific to male patients with AS, an effect that was not directly linked to levels of sex hormones[61]. Although the mechanism behind this male-specific effect is currently unclear, the implications are important: IL-17 inhibition in female patients might not be as effective as in male patients, and this difference should be carefully examined in ongoing clinical trials.

Box 1 | **Role of microbiome and mycobiome in AS**

Of the environmental triggers skewing type 3 immunity in ankylosing spondylitis (AS), the microbiome has received considerable attention (reviewed previously[104,105]). In patients with AS, dysbiosis has been observed in the gut microbiota[20], with abundance of the *Dialister* positively correlating with disease activity scores[106]. Studies in *HLA-B27*-transgenic rats support a role for HLA-B27 in skewing the host microbiota[107]. Microorganisms are an important pathogenic factor in animal models of spondyloarthritis (SpA), as germ-free *HLA-B27*-transgenic rats and SKG mice are disease-free[108,109]. *Chlamydia* infection in SKG mice promotes axial and peripheral arthritis, uveitis and psoriasis, but not colitis[110]. Furthermore, *Chlamydia*-induced disease in SKG mice is TNF-dependent, without a defined role for IL-17 (REF. 110). One striking feature of the SKG mouse model is that fungi are required to initiate a full spectrum of IL-17-dependent, SpA-like disease[111]. This feature is particularly relevant to AS, as fungal signalling through lectins and subsequent effector molecule induction involve a number of AS risk genes identified through genome-wide association studies, namely *CARD9*, *NFKBIA*, *TNFRSF1A* (encoding TNF receptor 1), *PTGER4*, *IL1R2*, *IL-6R* and, importantly, *IL-23R*[9]. Although several technical hurdles remain to be overcome in mycobiome studies, this subject should be prioritized in future research given its potential role in AS pathogenesis.

Current research on IL-17 in AS is focused on the identification of factors that induce IL-17 production and cell types that produce IL-17. Microbial dysbiosis might be a key driver of IL-17 induction in AS (BOX 1), which needs to be addressed in future studies. To date, the target cells of IL-17 in AS, or whether IL-17 is a key initiator of pathogenic processes, is not clear.

IL-17-producing cells. A multitude of IL-17-producing cells have been implicated in AS, including myeloid cells, adaptive lymphocytes, innate-like lymphocytes and innate lymphocytes[62] (FIG. 2). T_H17 cells are the most common class of IL-17-producing adaptive lymphocytes. In patients with AS, T_H17 cells are characterized by enhanced expression of KIR3DL2 (discussed above) and display an oligoclonal T cell receptor (TCR) repertoire[39]. A study published in 2016 implicated $IL\text{-}17^+$ $CD8^+$ T cells in AS[63]. The expression of T-bet (encoded by *TBX21*), a prototypic T helper 1 (T_H1) cell transcription factor identified through GWAS, was found to be elevated in $CD8^+$ T cells of patients with AS, and to be associated with an IL-17 response[62]. Furthermore, $Tbx21^{-/-}$ SKG mice did not develop SpA-like disease and showed a drastic reduction in the number of $IL\text{-}17^+$ $CD8^+$ T cells as compared with wild-type SKG mice. Currently, it is unclear how these findings in SKG mice are consistent with results obtained in mouse colitis models, which revealed that T-bet-deficient mice have unrestrained IL-17 production owing to the role of this transcription factor in suppressing IL-23 receptor (IL-23R) expression in T_H17 cells[64]. Furthermore, T-bet is essential in regulating the activity of $CD4^+$ T cells, especially in their transition from the lamina propria to the epithelium, during which they acquire CD8α expression and lose expression of forkhead box protein P3 (FOXP3) while maintaining a regulatory function[65].

Innate-like lymphocytes possess an invariant TCR, have limited clonality and are quickly activated upon stimulation. Examples of innate-like lymphocytes are mucosal-associated invariant T (MAIT) cells and γδ T cells. Human studies published in 2016 showed that MAIT cells from patients with AS produce higher levels of IL-17 compared with those from healthy individuals, and are enriched in the inflamed joint of patients with AS[66,67]. Elevated levels of IL-17 are attributed to priming of MAIT cells with IL-7; this phenomenon might extend to T_H17 cells and is important because *IL7R* confers AS susceptibility. Furthermore, IL-7 might prove to be a viable therapeutic target given that levels of this cytokine are elevated in inflamed gut and joint tissue from patients with AS[68,69].

The number of γδ T cells that produce IL-17 and express IL-23R are elevated in AS[70]. Although not yet confirmed in humans, $IL\text{-}17^+$ $CD4^-$ $CD8^-$ $IL\text{-}23R^+$ T cells are found in the enthesis of mice with IL-23-induced SpA-like disease[71]. Evidence from mouse studies suggests that these cells are tissue-resident $V\gamma6^+$ γδ T cells[72]. This particular subtype promotes bone growth through IL-17 (REF. 73), and is thus a putative pathogenic cell population linking IL-23-induced inflammation to bone growth in the enthesis. The synovio-entheseal complex is considered important in the

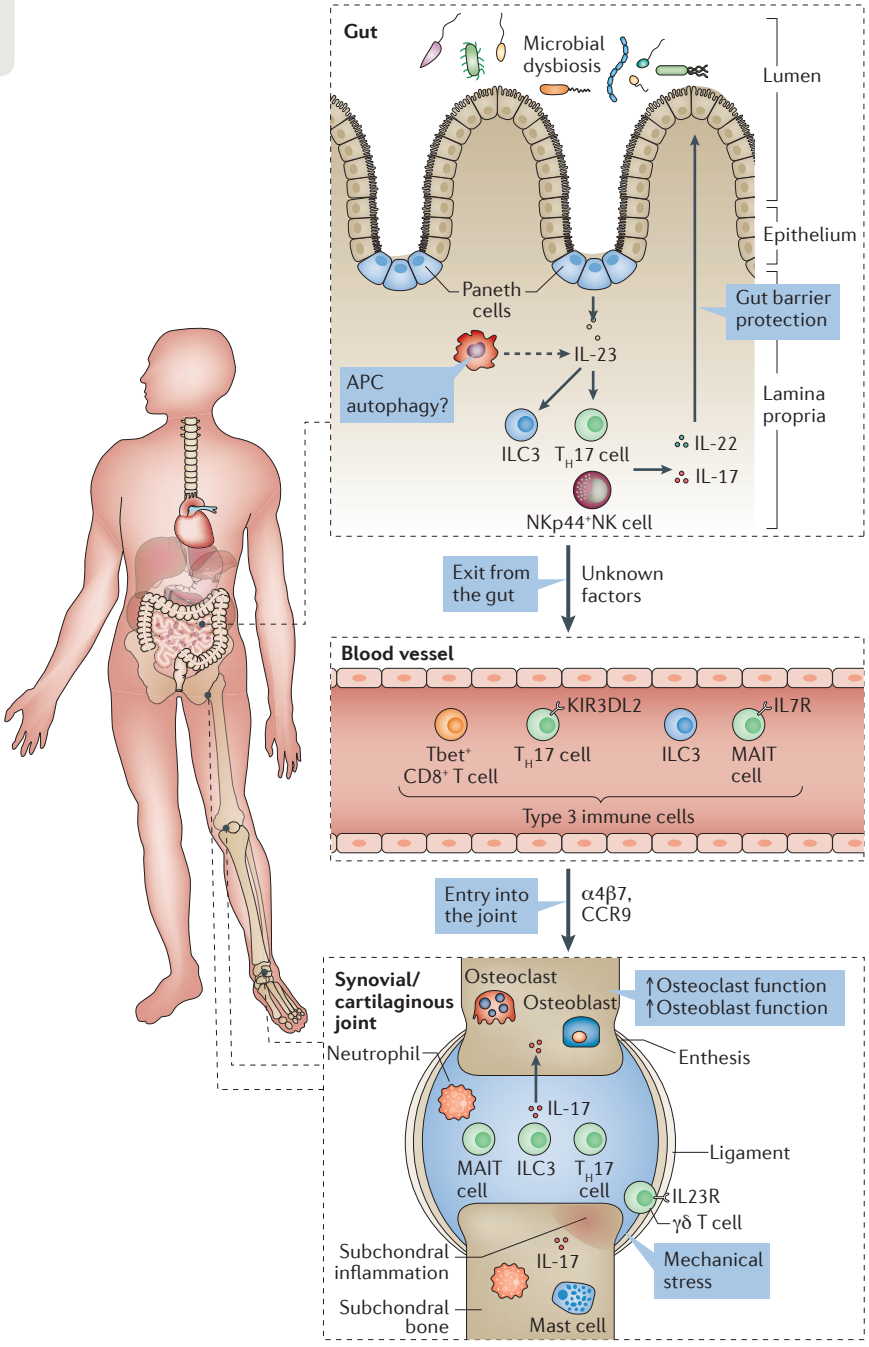

Figure 2 | **Type 3 immunity and AS.** Strong evidence exists for a mucosal origin of type 3 inflammation in patients with ankylosing spondylitis (AS). Paneth cells are an important source of IL-23, which acts on local innate and adaptive lymphocytes to stimulate an IL-17–IL-22 response. These cytokines are considered protective as they promote epithelium integrity. HLA-B27-influenced microbial dysbiosis might drive inflammatory events in the mucosa. IL-17-producing lymphocytes, probably of gut origin, are elevated in the blood of patients with AS. Gut-related trafficking markers, such as α4β7 and CC-chemokine receptor 9 (CCR9) have been implicated in the trafficking of type 3 immune cells into inflamed synovial tissue. Cells involved in type 3 immunity are enriched in different locations of synovial and cartilaginous joints of patients with AS, including synovial fluid, ligaments and subchondral bone marrow. Mechanical stress might drive, in part. the activity of such cells at the enthesis. IL-17 is known to alter the activity of osteoclasts and osteoblasts, probably contributing to aberrant bone formation. APC, antigen-presenting cell; IL7R , interleukin 7 receptor subunit α; ILC3; group 3 innate lymphoid cell; KIR3DL2, immunoglobulin-like receptor 3DL2; MAIT cell, mucosal-associated invariant T cell; NK cell, natural killer cell; T_H17 cell, T helper 17 cell.

pathogenesis of SpA[74,75], and mechanical loading of the entheses has been shown to activate inflammation and bone formation in the $TNF^{\Delta ARE}$ mouse model of SpA[76].

Innate lymphocytes, such as innate lymphoid cells (ILCs), do not have an adaptive immune receptor (TCR or B cell reptor (BCR)) and respond rapidly to stimulation. NK cells are the most common type of group 1 ILCs in humans and mice. Renewed interest in the function of NK cells in AS was sparked by the identification of $NKp44^+$ NK cell expansion in ileal tissues from patients with AS, which represent a major source of IL-22, a cytokine that acts in concert with IL-17 in mediating effector functions[77]. Similarly, group 3 ILCs (ILC3s) that produce IL-17 and IL-22 are expanded in the peripheral blood and tissues from patients with AS[68].

Thus, IL-17 upregulation in patients with AS is evidently not cell-specific, and probably reflects a global dysregulation of IL-17-producing type 3 immune cells (TABLE 1). The relative contribution to AS of IL-17 derived from various type 3 immune cells has yet to be determined, but might be tissue-specific. It is unclear whether all type 3 immune cells are pathogenic. Indeed, ILC3s seem to target commensal-specific T cells and thus to promote tolerance to gut microorganisms[78], whereas IL-17 produced by intestinal γδ T cells is essential for epithelial integrity[79].

T_H17 cell plasticity. It is becoming clear that not all T_H17 cells are 'created equal', and a large body of work indicates an inherent instability of some T_H17 cell populations, especially in the gut[80,81]. Despite T_H17 cells being a focus of AS research, their instability has not been investigated. A decade ago, T_H17 cells were found to require transforming growth factor-β (TGFβ) and IL-6 to differentiate from naive T cells[82,83]. Soon afterwards, IL-23 was found to be crucial in stabilizing the T_H17 cell phenotype and promoting T_H17 cell pathogenicity in autoimmune settings[84]. Extensive fate-mapping studies published over the past year provide conclusive evidence of, and mechanistic insight into, T_H17 cell instability[85,86,87]; strikingly, this instability is very common in T_H17 cells of the intestinal lamina propria, where up to half of IL-17-producing $CD4^+$ T cells stop expressing this cytokine during resolution of inflammation[85]. Furthermore, two studies reported the differentiation of T_H17 cells to regulatory T (T_{reg}) cells expressing IL-10, FOXP3 or both[86,87]. Exposure to microbial antigens through antigen-presenting cells is essential for the transition of T_H17 cells into T_{reg} cells[87], with cytokines such as TGFβ promoting T_H17 cell destabilization[85].

Data on the role of IL-23 in the generation of 'ex-T_H17' T_{reg} cells are conflicting. Some studies have shown that IL-23 promotes the accumulation of T_{reg} cells in the gut[88], which are probably 'ex-T_H17' T_{reg} cells[87]. Conversely, a 2016 study showed that IL-23 promotes the stability of pathogenic T_H17 cells through the transcription factor PR domain zinc finger protein 1 (PRDM1)[89]. This finding is particularly important given the association of PRDM1 with Crohn's disease[90]. The implication of these findings is that the increased number of T_H17 cells in patients with AS might not result from preferential

Synovio-enthesal complex
Anatomical unit comprising the fibrous insertion of tendon or ligament enthesis and the adjacent synovial membrane of the bursa.

Fate-mapping studies
Studies that investigate the origin of cell populations through labelling and tracking of cells of interest. Immunological studies typically use membrane-incorporated dyes, or genetic switches that result in constitutive fluorochrome expression if a cell marker is expressed.

differation of naive T cells, but rather through a reduced plasticity of mature T_H17 cells. The instability of T_H17 cells and their transition to a non-pathogenic phenotype should be investigated in future studies of AS.

Targeting type 3 immunity in AS. Given its prominent role in AS, type 3 immunity is an important therapeutic target. A multitude of type 3 immunity targets have been tested, including upstream inducers of type 3 immune cells and downstream effector molecules. Although TNF inhibitors are very effective in controlling AS symptoms, connecting this response to effects on type 3 immunity is difficult. In patients with AS, although TNF inhibition seems to reduce the number of neutrophils accumulating in the synovium and the number of ILC3s in the gut[68,91], and to suppress *IL17R* expression in blood cells[92], it does not seem to affect serum levels of IL-23 or prostaglandin E_2 (PGE_2)[93]. A 2014 study showed that TNF inhibition promotes IL-10 production in human T_H17 cells[94]. Whether a similar mechanism occurs in patients with AS is not clear.

Treatment strategies that might affect T_H17 differentiation have had mixed results in the clinic. IL-6 seemed to be a promising target in AS owing to its increased expression in patients and its role in T_H17 cell development. However, clinical trials of IL-6 inhibition in patients with AS were terminated as the primary outcome of clinical response (according to Assessment in Ankylosing Spondylitis International Working Group criteria for 20% improvement) was not reached, despite a substantial reduction in C-reactive protein levels[95,96]. This result could reflect the relatively lower activity of IL-6 in the development of human T_H17 cells compared with that of mouse cells[97]. Conversely, a proof-of-concept study suggested that IL-12 and IL-23 blockade with ustekinumab is effective in patients with AS[98]. The specific interleukin receptor that accounts for this response remains to be defined.

Rheumatologists might have unintentionally targeted type 3 immunity for decades with NSAIDs. The AS risk gene *PTGER4*, which encodes PGE_2 receptor EP4 subtype, is expressed on, and promotes pathogenicity of, T_H17 cells[99]. A subset of patients with AS respond very well to treatment with NSAIDs, which act by suppressing prostaglandin production. A biomarker for predicting response, such as PTGER4 polymorphism, could prove to be useful in the targeted treatment of these patients.

A number of inhibitors of type 3 immunity are currently being tested and might be useful in the treatment of AS. As discussed above, ERAP1 inhibition reduces T_H17 cell expansion and IL-17 release[44]. Small molecules targeting RORγt seem to be effective at blocking IL-17-dependent colitis in mice and *in vitro* differentiation of T_H17 cells from patients with AS[100,101]. Importantly, RORγt inhibition in mice with colitis and gut explants derived from patients with colitis selectively inhibited pathogenic T_H17 cells but not protective ILC3s.

To date, the only type 3 immunity cytokine that has been successfully targeted in AS is IL-17. In phase III clinical trials, secukinumab (an anti-IL-17 monoclonal antibody) was shown to be as effective as TNF inhibitors in patients with AS[3]. Conversely, secukinumab failed to reduce severity of Crohn's disease in a clinical trial, with results suggesting that this drug exacerbates colitis[102]. These results probably reflect the tissue-specific roles of IL-17, which are protective in the gut and detrimental in the joint[79]. Although these data have caused concern for the treatment of patients with AS who might have subclinical gut inflammation, data from other secukinumab trials in patients with psoriasis, psoriatic arthritis and AS are promising as they do not show a considerable increase in *de novo* IBD development[103]. Careful monitoring of patients with AS treated with secukinumab will be essential in ongoing clinical trials.

Conclusions

The progress in our understanding of AS pathogenesis has been remarkably rapid. Four decades have passed since the discovery of the *HLA-B27*–AS association, with an accumulation of substantial, incremental knowledge; however, paradigm-shifting evidence demonstrating exactly how this gene confers susceptibility to AS has yet to emerge. High-throughput techniques and a renewed enthusiasm for functional studies are contributing to advance our understanding of AS. Multiple interacting proteins involved in the antigen-presentation pathway, including ERAP1, ERAP2, HLA-B27 and SEC16A, have been found to be associated with AS. The role of type 3 immunity is increasingly recognized and future studies should help identify crucial cell types involved in the disease. Patients with AS will benefit from the understanding of signalling pathways involved in AS pathogenesis and the differential regulation of TNF and IL-17 signalling pathways in personalized medicine approaches.

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All the authors researched the data for the article, made substantial contributions to discussion of content, wrote the article, and edited/reviewed the manuscript before submission. V.R. and E.G. contributed equally to this work.

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