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## Lessons on SpA Pathogenesis from Animal Models

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## Abstract

Understanding the complex mechanisms underlying a disorder such as spondyloarthritis (SpA) may benefit from studying animal models. Several suitable models have been developed, in particular to investigate the role of genetic factors predisposing to SpA, including HLA-B27, *ERAP1* and genes related to the interleukin (IL)-23/IL-17 axis. One of the best examples of such research is the HLA-B27 transgenic rat model that fostered the emergence of original theories regarding HLA-B27 pathogenicity, including dysregulation of innate immunity, contribution of the adaptive immune system to chronic inflammation and influence of the microbiota on disease development. Very recently, a new model of HLA-B27 transgenic *Drosophila* helped to expand further some of those theories in an unexpected direction involving the TGF $\beta$ /BMP family of mediators. On the other hand, several spontaneous, inducible and/or genetically modified mouse models - including SKG mouse, TNF<sup>Aare</sup> mouse and IL-23-inducible mouse model of SpA- have highlighted the importance of TNF $\alpha$  and IL-23/IL-17 axis in the development of SpA manifestations. Altogether, those animal models afford not only to study disease mechanism but also to investigate putative therapeutic targets.

**Keywords:** Ankylosing spondylitis, spondyloarthritis, animal models, HLA-B27, TNF $\alpha$ ; Interleukin-23; Interleukin-17.

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## Introduction

Spondyloarthritis (SpA) is one of the most common groups of chronic inflammatory joint disorders, affecting between 0.2% and 1.61% of the general adult population [1, 2]. The hallmark of SpA is articular inflammation which may affect both axial (i.e. spinal, pelvic and thoracic wall joints) and peripheral skeleton structures, including arthritis, enthesitis and dactylitis. In addition, the spectrum of SpA comprises frequent extra-articular features, including anterior uveitis, psoriasis, and inflammatory bowel disease (IBD, i.e. Crohn's disease (CD) or ulcerative colitis (UC)). A major characteristic of SpA is the variability of its clinical presentation. This led initially to the recognition of distinct entities which were separated altogether from rheumatoid arthritis as "seronegative" spondylarthritides, given their lack of association with autoantibodies, such as rheumatoid factor or anti-citrullinated peptides antibodies. They comprise ankylosing spondylitis (AS), psoriatic arthritis (PsA), reactive arthritis (ReA) and arthritis associated with idiopathic inflammatory bowel diseases (AIBD). Their grouping as SpA was further proposed, based on the observation of frequent overlap between these disorders, that may arise consecutively in the same patient (e.g. AS "secondary" to PsA, ReA, or AIBD), be virtually indistinguishable from each other or affect different individuals within a same family [3, 4]. Classification criteria were then developed to account for the unified concept of SpA [4].

Another well-established characteristic of SpA is its strong heritability that is in large part explained by its association with the major histocompatibility (MHC) antigen HLA-B27, discovered 47 years ago [5, 6]. However, the MHC region itself has been estimated to account for merely half of the whole genetic predisposition [7]. Indeed, over the last decade, multiple polymorphisms associated with increased disease susceptibility have been identified in genes outside of the MHC [8]. Among them, two closely related endoplasmic reticulum (ER) aminopeptidases genes, *ERAP1* and *ERAP2* are of particular interest, as they may influence HLA-B27 effect [9]. Such interpretation is supported by the evidence for genetic interaction between *ERAP1* and *HLAB* loci in determining disease susceptibility [10]. Moreover, several other disease-associated genes code for cytokines, their receptors or downstream effectors that are involved in the type 3 immune response, including *IL12B*, *IL27A*, *IL1R2*, *IL6R*, *IL23R*, *JAK2*, *TYK2*, *STAT3* and *CARD9* [8]. Thus, such wealth of recent findings opened new tracks for mechanistic research on SpA pathophysiology. Noteworthy however, the contribution of each of these new *loci* to the whole genetic susceptibility is relatively sparse, especially as

compared to that of HLA-B27. Therefore, trying to explain the predisposing role of HLA-B27 in SpA has remained a major focus of research in the field for decades.

### **Animal models have improved our understanding of HLA-B27 implication in SpA**

The association between HLA-B27 and SpA is one of the first described between an MHC allele and a human disorder. However, despite its remarkable strength and consistency across populations, there is currently no consensus on its significance and the role played by this HLA molecule in disease pathogenesis remains largely unexplained.

HLA-B27 is a protein encoded by the most polymorphic human gene., i.e. the *HLAB locus*. As for other classical class I MHC molecule, its main function is to bind short peptides spanning 8 to 10 amino acids in large in and to present them in combination with the non-covalently-associated invariant light chain,  $\beta$ 2-microglobulin ( $\beta$ 2m), at the cell surface, where the trimolecular complex is specifically recognized by CD8+ T cells. The allelic diversity of HLA-B molecules mainly concerns their peptide-binding groove, resulting in distinctive capacities to bind defined peptides. Thus, the collection of peptides that a given HLA-B molecule could bind and present -the peptidome- is dictated by its particular structure. This has been clearly shown in the case of HLA-B27, which selectively presents peptides bearing an Arg residue as a second anchor position, a quite unique property among class I MHC alleles [11]. Thus, the specificity of its antigen-presenting function is at the origin of one of the earliest theories regarding HLA-B27 pathogenicity, the "arthritogenic peptide" hypothesis which speculates on an auto-immune attack driven by cytotoxic CD8+ T cells and targeting a self-peptide exquisitely presented by HLA-B27 [12]. Complementary to the foregoing, as a possible explanation for breaking of tolerance, immune response initiated against an infectious agent could secondarily shift to self-antigen attack by virtue of molecular mimicry. This could notably explain occurrence of ReA in the weeks following an intestinal or urogenital bacterial infection [13]. Several arguments indirectly support those theories, including evidence for recurrent CD8+ T cell clonotypes and for cytotoxic CD8+ T cell lines displaying cross-reactivity for Epstein-Barr viral epitope and vasointestinal peptide receptor-derived self-peptide, in HLA-B27+ SpA patients [14, 15]. Nevertheless, the pathogenicity of such CD8+ T cells remains to be demonstrated. The lack of proof for a direct implication of the canonical antigen-presenting function of HLA-B27 in its pathogenicity fostered the emergence of alternative theories based on peculiar biochemical behaviors of the molecule. The most popular alternate hypotheses proposed over the last two decades speculated either on a

remarkable propension of the HLA-B27 heavy chain to misfold during its synthesis in the endoplasmic reticulum (ER) and to trigger an unfolded protein response (UPR) or on the formation of aberrant heavy chains homodimers expressed at the surface of the cell where they may interact with killer immunoglobulin-like receptors (KIR) expressed by natural killer (NK) cells and CD4<sup>+</sup> T cells, both of which could trigger an inflammatory response [16, 17].

#### *HLA-B27 transgenic rat model of SpA*

Several lines of HLA-B27 transgenic mice were initially produced that remained healthy, except for the development of arthritis when crossed with  $\beta$ 2m-deficient mice [18]. However, such phenotype appeared later not to be specific for the HLA-B27 transgene but rather for the mouse genetic background [19].

In contrast, several lines of HLA-B27/human  $\beta$ 2m (h $\beta$ 2m) double transgenic lines of rats develop a spontaneous phenotype that resembles SpA (rat-SpA). This striking observation established in the early 90's has remained until now the strongest argument supporting a direct implication of HLA-B27 itself -rather than nearby genetic factor- in the pathogenesis of SpA [20]. The effect of HLA-B27 is overwhelming since it is observed on several genetic backgrounds and in different lines of rats harboring distinct HLA-B27/h $\beta$ 2m transgenic *loci* (each *locus* consisting in various copy numbers of both transgenes inserted in a unique genomic site). It is also specific since it is not observed in a rat line transgenic for HLA-B7/h $\beta$ 2m control allele, structurally close to HLA-B27 and negatively associated with SpA [10]. Importantly, it is dependent on a large number of copies of both HLA-B27 and h $\beta$ 2m transgenes. Hence, the respective numbers of each transgene -dictating the amount of the corresponding transcript/protein that is produced- condition the phenotypic expression of rat-SpA [21]. In lines bearing the highest numbers of copies of HLA-B27 transgene, the phenotype affects both sexes and mimics severe form of AIBD, by combining peripheral arthritis -more prevalent in males- with UC and psoriatic skin changes. Moreover, increasing the number of h $\beta$ 2m transgene copies resulted in heightened prevalence and severity of arthritis [22, 23]. Other line with fewer copies of HLA-B27 but a high number of h $\beta$ 2m transgene copies develop a phenotype more reminiscent of AS, restricted to males and to osteo-articular tissue, including spinal and sacroiliac joint inflammation with osteoproliferation, in addition to peripheral arthritis [23, 24]. In both cases, males uniformly

develop early epididymo-orchitis that seems to be a critical event, at least for the second phenotype since orchietomy prevents its development [25].

Several key experiments have allowed to identify major factors driving spontaneous SpA development in this HLA-B27 transgenic rat model and their conclusions have significantly influenced our understanding of HLA-B27 pathogenicity.

*a- The critical implication of myeloid-derived antigen-presenting cells in rat-SpA*

Rat-SpA could be induced in irradiated wild-type rats by the transfer of immature haematopoietic stem cells originating from the bone marrow or fetal liver from disease-prone HLA-B27 transgenic donor rats, but not by mature lymphocytes contained in lymph nodes or spleen. Reciprocally, HLA-B27 transgenic rats were cured from established disease by irradiation followed by transplantation of bone marrow cells from wild-type donor [26]. This indicated that expression of the HLA-B27/hβ2m transgene in myeloid-derived cell -suspected to be an antigen-presenting cell (APC)- was necessary and sufficient to induce rat-SpA. This observation fostered an extensive characterization of APCs -with a particular interest for conventional dendritic cells (cDCs), which are potent professional APCs bridging innate to adaptive immunity- evidencing functional alterations that could contribute to disease pathogenesis. First of all, APCs from disease-prone HLA-B27 transgenic rats showed a decreased capacity to stimulate T cells -even in antigen-independent assays- and this correlated strongly with disease susceptibility in a wide variety of transgenic lines [27–29]. Contrasting with such functional impairment, cDCs from HLA-B27 transgenic rats are prone to drive a T-helper 17 (Th17) bias during CD4<sup>+</sup> T cell differentiation and/or activation, an observation consistent with the accumulation of Th17 cells seen in this model [29–31]. A consistent biased impact was also observed on regulatory T cells (Treg) that produced more IL-17A and less IL-10 when activated with DCs from HLA-B27 transgenic rat [32]. At the molecular level, HLA-B27 transgenic DCs displayed altered cytoskeleton dynamic and were impaired in their capacity to engage costimulatory molecules -in particular CD28- and to form a functional immunological synapse with T cells [27, 33, 34]. This could contribute to both the stimulatory inefficacy and the Th17 bias -given that CD86-CD28 signaling impairment seems to favor Th17 bias [35].

Looking in more details at cDC subpopulations, an imbalance was found between the cDC2 subset -implicated in T cell activation and cytokines production- and cDC1 -critical for

tolerance induction- marked by a decreased proportion of the latter in the spleen, the mesenteric lymph nodes and the lymph draining the gut [29, 36]. This could at least in part be related to the defective migration of cDC1 from the colonic mucosa to mesenteric lymph nodes, possibly due to cytoskeleton alteration [36]. Of particular interest was a relative decrease in all the organs studied and an altered capacity to support *in vitro* Treg differentiation, of the XCR1+ cDC1 subset, known to promote the induction of peripheral Treg by the production of transforming growth factor- $\beta$ 1 (TGF $\beta$ 1) and retinoic acid from vitamin A [36, 37]. Besides, the cDC2 subset from disease-prone HLA-B27 transgenic lines displayed a down-regulation of *Interferon gamma* (*Ifng*) and IFN-regulated genes at the transcriptomic level that could be secondary to an induction of *suppressor of cytokine signaling* (*Socs*)3 gene. Interestingly, such "reverse" IFN signature appeared to be shared with APCs from AS patients [38, 39]. As predicted, down-regulation of the IFN $\gamma$  pathway in B27 transgenic rat cDC2 was associated with a decreased expression of IL-27, a cytokine belonging to the IL-12 family and known to oppose IL-17 and to favor IL-10 production from T cells, as well as Treg activation [38, 40]. IL-10 expression by cDC2 was concomitantly decreased, potentially contributing to a pro-inflammatory profile [38].

#### *b- The pathogenic role of CD4+ T cells in rat-SpA*

Rat-SpA fails to develop in athymic nude HLA-B27 transgenic rats, unless reconstituted with T cells. This indicated that thymically-derived T cells are required for disease expression [41]. The transfer of purified CD4+ T cells to nude B27 rats, even from disease-resistant donors, was associated with vigorous disease induction, whereas CD8+ T cells were relatively inefficient [41]. Importantly, rat-SpA was unaffected by CD8+ T cells depletion [42]. The foregoing results obtained in lines developing the AIBD phenotype are consistent with the observation that both phenotypes of rat-SpA remained unchanged in rats lacking the CD8 molecule, despite a profound alteration of their capacity to raise a cytotoxic CD8+ T cell-mediated response [43]. Additionally, HLA-B27 transgenic rats were shown as defective in their capacity to mount a CD8+ T cell-mediated cytotoxic response [44 and Breban M., unpublished observation], an observation possibly related to altered XCR1+ cDC1 subset. Altogether, those observations do not support the implication of canonical antigen-presenting function of HLA-B27 to CD8+ T cells as the mechanism of its pathogenicity and appear therefore inconsistent with the "arthritisgenic peptide" hypothesis.



Concerning the nature of pathogenic CD4<sup>+</sup> T cells, Th17 cells are the most likely involved, considering the selective expansion of those cells concomitant with disease development, as mentioned above [30]. Nevertheless, anti-IL-17 antibody treatment was inefficient at preventing disease onset in a rat line developing the AIBD phenotype [30]. This suggests the critical involvement of other pro-inflammatory mediator(s) among which TNF $\alpha$  is a compelling candidate, since APCs from HLA-B27 transgenic rat boost its production by CD4<sup>+</sup> T cells and it is co-expressed by Th17 cells [30]. Consistently, anti-TNF treatment was effective in reducing gut and joint manifestations in HLA-B27 transgenic rat [45].

*c- Gut and joint inflammation are dependent on the gut microbiota*

The role of gut microbiota in HLA-B27 transgenic rat has recently been reviewed elsewhere and will only be summarized herein [46]. When raised under germ-free conditions, HLA-B27 transgenic rats from the lines developing an AIBD phenotype in conventional conditions, were protected from gut and joint inflammation, both of which were triggered upon recolonization with commensal microbiota. This showed the dependence of SpA manifestations on the presence of regular microbiota. Selective recolonization of the germ-free rats showed that strictly anaerobic bacteria, especially *Bacteroides spp.* (and particularly *B. vulgatus*) were the most pro-inflammatory but without absolute specificity [47]. The triggering of gut inflammation in germ-free HLA-B27 transgenic rats mono-associated with distinct commensal bacteria indicated that expression of HLA-B27 was facilitating an aberrant pro-inflammatory response to various resident bacteria. In line with such interpretation, APCs from HLA-B27 transgenic rats exhibited decreased IL-10 production in response to Toll-like receptor (TLR) stimuli, that could contribute to exaggerate the immune response to bacterial components. Indeed, CD4<sup>+</sup> T cells from those rats were overproducing IFN- $\gamma$  in response to antigens derived from commensal enteric micro-organisms, suggesting a loss of tolerance towards regular microbiota [48–50]. Additionally, dysregulated immune response in HLA-B27 transgenic rats could contribute to disease predisposition by favoring an imbalance between pro-inflammatory and anti-inflammatory microbiota. In favor of such possibility, microbiota composition is altered in the gut from HLA-B27 transgenic rats -a situation referred to as dysbiosis. However, the implication of this finding remains difficult to interpret, since the nature of dysbiosis appears fairly variable, depending on the rat genetic background and/or the animal facility, with little overlap between different disease-prone strains [51, 52].

The foregoing results were obtained in rat lines developing an AIBD phenotype in which gut inflammation may strongly influence dysbiosis and reciprocally, but the influence of gut microbiota has not been directly examined in the line developing an isolated articular phenotype. However, splenocytes from the latter line were shown to overproduce pro-inflammatory cytokines -i.e.  $\text{TNF}\alpha$ , IL-1, and IL-6- in response to TLR and/or dectin-1 ligands. Furthermore, in this line, arthritis and spondylitis could be triggered by using suboptimal dose of Freund's adjuvant -an emulsion of heat-killed *Mycobacterium tuberculosis*-, showing again that HLA-B27 expression amplifies the immune response towards stimuli of microbial origin [53].

#### *d- Involvement of non-canonical molecular behavior of HLA-B27 in rat-SpA*

The above results indicate that HLA-B27/h $\beta$ 2m expression may drive rat-SpA by dysregulating APCs function, resulting in biased CD4+ T cell response to stimuli, such as microbial components. This happens in conditions of high expression levels of both transgenes. However, the precise molecular mechanism by which HLA-B27 expression may affect APC biology remains an unsolved issue.

The tendency of HLA-B27 to misfold in the ER could be a critical event, by triggering a UPR, leading to type 3 immune response driven by IL-23 production [54]. Indeed, such consequences have been shown to happen in bone marrow-derived macrophages from HLA-B27 transgenic rats, upon LPS activation [31, 55]. Other experiments carried with mature cDCs revealed also some possible consequences of a UPR, such as increased expression of ER Protein-disulfide isomerases A3, A4 and A6 [33, 38]. Therefore, experiments were carried out to functionally test UPR implication. Their principle was to facilitate HLA-B27 folding by increasing h $\beta$ 2m expression, using genetic cross with a line carrying a multicopy h $\beta$ 2m transgenic *locus*. As expected, the result was a decrease of UPR markers, but contrary to the prediction that UPR was a triggering event in rat-SpA, disease was not attenuated. Even more, both severity and frequency of arthritis worsened -as already mentioned above [23, 38]. Therefore, UPR may not be the critical molecular mechanism by which HLA-B27 triggers rat-SpA.

One of the remarkable non-canonical features of HLA-B27 is its propensity to form homodimers/oligomers of heavy chain [56]. Intriguingly, despite reducing UPR, increasing h $\beta$ 2m level in HLA-B27 transgenic rats favored an intracellular accumulation of misfolded

HLA-B27 heavy chain in combination with h $\beta$ 2m and ER chaperones, i.e. BiP and Glucose regulated protein-94, in ER-derived vesicles [57]. Similar phenomenon was observed in human cell line transfected with HLA-B27 subtypes differently associated with AS predisposition, the extent of which correlated positively with disease predisposition. It was also observed in lymphoid cells from SpA patients in greater amount than in cells from control individuals [57]. Such accumulation could possibly be due to an impaired ubiquitination of HLA-B27 heavy chain dimers/oligomers, a process that facilitates the disposal of misfolded protein [58]. How such phenomenon could contribute to cellular disturbance is still unclear. However, it indicates that the pathogenicity of HLA-B27 may be due to an accumulation of unusual conformers, likely devoid of canonical peptide, in conjunction with  $\beta$ 2m and ER chaperones. With this regard, it is interesting that the peptidome of HLA-B27 transgenic rats did not differ between disease-susceptible and healthy lines and that a deletion of Erp1 -an ER aminopeptidase involved in generating peptides suitable for class I MHC loading- did not affect rat-SpA expression despite a marked impact on HLA-B27 peptidome [59]. This fits with the hypothesis that well-folded HLA-B27 molecules bound to canonical peptides are not involved in disease pathogenesis.

Homodimers/oligomers of HLA-B27 heavy chain have also been detected at the surface of human or HLA-B27 transgenic rat cells [56, 60, 61]. These non-canonical conformers could bind to innate immune receptors that include KIRs on human cells and paired immunoglobulin receptors expressed by rodent APCs, with putative pro-inflammatory consequences, including TNF $\alpha$  production [16, 60]. However, the relevance of this phenomenon to HLA-B27 pathogenicity has recently been challenged, given its lack of correlation with disease susceptibility among HLA-B27 subtypes [62].

#### *HLA-B27 transgenic Drosophila, a new model to study HLA-B27 pathogenicity*

Deciphering the non-canonical consequences of HLA-B27 expression could benefit from a simplified model. This was the major reason to use *Drosophila*, an organism originally devoid of MHC molecule and of several key elements of the class I MHC peptide-loading complex, to further address such issue [63]. Several HLA-B transgenes were inserted as single copy in a predefined *Drosophila* genomic site. They were expressed either alone or in combination with h $\beta$ 2m, using tissue-specific drivers. Two distinct SpA-associated HLA-B27 subtypes, i.e. HLA-B\*27:04 and HLA-B\*27:05 were studied in comparison with the SpA-negatively-associated HLA-B\*07:02 allele.

Remarkably, when expressed in the wing or eye, both SpA-associated HLA-B27 transgenes induced striking phenotypes, i.e. a loss of the crossveins and a small-eye, respectively [63]. Those phenotypes required the presence of h $\beta$ 2m and were absent in the HLA-B\*07:02 transgenic *Drosophila*. Noteworthy, in the presence of h $\beta$ 2m, HLA-B27 conformers, presumably loaded with peptides (as recognized by ME1 antibody, a murine monoclonal antibody specific for well-folded HLA-B27 and B7 molecules) were expressed at the cell surface despite the lack of peptide-loading complex, consistently with previous observation made in *Drosophila* cell line [63, 64].

Crossing the HLA-B27 transgenic *Drosophila* line with mutant flies or flies which misexpress specific gene, then analyzing phenotypes of offspring, allowed to pinpoint affected signaling pathway responsible for the observed phenotypes. They showed that HLA-B27 expression interfered with bone morphogenetic protein (BMP) signaling, a developmental pathway involved in the formation of *Drosophila* wing and eye. More specifically, HLA-B27 exerted a dominant negative effect on the type I BMP receptor (BMPRI) Saxophone. Saxophone, like its mammalian orthologs, activin receptor-like kinase 1 (ALK1) and ALK2 associates with other BMPRI and type II BMP receptors to form heterotetramer. In this complex, they can exert either agonistic or antagonistic effect on signaling, depending on the context [65]. In *Drosophila*, expression of the HLA-B27/h $\beta$ 2m transgenes, resulted in a loss of the antagonistic function of Saxophone on BMP signaling. Consistently, physical interaction was shown at the cell surface between folded HLA-B27/h $\beta$ 2m complexes and Saxophone [63].

Thus, the HLA-B27 transgenic *Drosophila* model unraveled a novel molecular mechanism that could explain HLA-B27 pathogenicity. Accordingly, HLA-B27 may exert a dominant negative effect on ALK1 and/or ALK2, that could be relevant to human SpA. Those receptors are involved in the negative regulation of BMP and Activin//transforming growth factor  $\beta$  (TGF $\beta$ ) signaling in mammals and their inhibition may result in excessive ossification and inflammation, two hallmarks of SpA. Indeed, excessive ossification in response to Activin is characteristic of *fibrodysplasia ossificans progressiva*, a rare autosomal dominant mendelian disorder due to mutations of *ALK2* that reverse its inhibitory function [66].

Those intriguing results prompted us to examine whether they could be extrapolated to cells from SpA patients. Indeed, a close physical interaction was evidenced between well-folded HLA-B27 molecules and ALK2 at the surface of B-lymphoblastoid cell lines from AS

patients, that was not detected with other HLA-B molecules in control lines [63]. Moreover, increased signaling in response to Activin-A and TGF $\beta$  -two ligands that may be antagonized by ALK1 or ALK2- was shown in T cells from HLA-B27+ SpA patients, as compared to control subjects [63].

#### *How ERAPs polymorphisms may influence SpA susceptibility*

Several polymorphisms of *ERAP1* and *ERAP2* genes affect SpA susceptibility [10, 67, 68]. The products of these two neighboring genes are structurally related aminopeptidases located in the ER, where they trim peptides to an optimal length for binding to class I MHC molecules [69, 70]. The association of *ERAP1* with SpA is conditional on HLA-B allele, principally HLA-B27, whereas this is not the case for *ERAP2* which is disease-associated independently of HLA-B27 status [10, 68]. Functionally, the *ERAP1* disease-associated alleles confer increased enzymatic activity, generating peptides with lower affinity for HLA-B27, which could favor their dissociation and contribute to the accumulation of misfolded HLA-B heavy chain in the ER-derived vesicles described above [59, 71]. As for *ERAP1*, SpA risk is favored by *ERAP2* polymorphisms conferring increased enzymatic activity, albeit their role may not be identical, given the lack of interaction with HLA-B27 [70].

An implication of ERAP1 in SpA, independent of HLA-B27, was suggested by recent study of an *ERAP1*<sup>-/-</sup> mouse model -of note, mouse is devoid of ERAP2- that develops several features of AS, including inflammation of spine, bony ankylosis of the lumbo-sacral joint, erosions of the sacroiliac joint, osteoporosis, dysbiosis and increased susceptibility to colitis [72]. This phenotype could be related to a decrease of tolerogenic DC and CD3+CD4+Foxp3-IL10+ Tr1-like cells, an inducible regulatory T cell population. Noteworthy, this observation does not corroborate the increased aminopeptidase activity conferred by ERAP1 polymorphisms associated with SpA in human, nor their interaction with HLA-B27. It could rather be linked to a decreased expression by tolerogenic DC and macrophages of Qa-2, the murine ortholog of HLA-G, a non-classical class I MHC molecule, thought to be important for Tr1 induction *via* its interaction with Ig-like transcript 2 (ILT-2) and ILT-4 receptors on naïve CD4+ T cells.

#### **Animal models addressing the role of cytokine signaling pathways in SpA**

*TNF $\alpha$ , a key cytokine in SpA*

The tremendous efficacy of anti-TNF $\alpha$  therapies established the critical role of this pro-inflammatory cytokine in SpA [73]. Its cellular source and targets in the context of SpA need to be characterized. Mouse models of deregulated TNF $\alpha$  production helped to clarify those questions, in addition to the HLA-B27 transgenic rat model discussed above, in which CD4 $^{+}$  T cells were shown to over-produce TNF $\alpha$ .

*a- The TNF $^{\Delta ARE}$  mouse*

Deletion of the adenylate-uridylate-rich elements in the murine TNF $\alpha$  locus (TNF $^{\Delta ARE}$  mice) leads to spontaneous and inducible overexpression of mouse TNF $\alpha$  by stromal cells and hemopoietic cells, including macrophages, T and B cells [74]. Those mice develop a spontaneous phenotype reminiscent of SpA by combining a destructive polyarthritis with sacroiliitis, enthesitis and a CD-like ileitis [74, 75]. Joint and gut inflammation exhibited both common and distinct requirements [75, 76]. Regarding IBD, TNF $\alpha$  produced by myeloid cells or T cells -but not B cell, nor stromal cells- exhibited pathogenic capacity and both hematopoietic and tissue stromal cells were equally important and sufficient targets for pathogenic TNF $\alpha$ . Ultimately, IBD resulted from an IL-12- and IFN- $\gamma$ -driven Th1-like response and on the activation of pathogenic CD8 $^{+}$  T cell, whereas CD4 $^{+}$  T cells were protective [76]. In contrast, adaptive immunity was dispensable for the induction of arthritis whereas tissue stroma-residing cells were required TNF $\alpha$  targets [74, 76, 77].

*b- The tmTNF-overexpressing mouse*

Biologically active TNF $\alpha$  exists either in a secreted form or as a transmembrane (tm)-bound cytokine, the former being released upon cleavage of the latter by ADAM17 (A disintegrin and metalloproteinase 17, also known as TNF converting enzyme, TACE). In SpA, tmTNF appears to be more expressed in the synovial membrane than in rheumatoid arthritis, and this could be related to lower ADAM17 activity, raising the intriguing possibility that tmTNF could be specially involved in SpA [78]. Indeed, the *TgA86* mouse that overexpresses tmTNF -due to defective ADAM17- develops axial and peripheral joint synovitis, enthesitis, and osteitis, with endochondral new bone formation ultimately leading to complete axial and peripheral joint ankylosis, typical of SpA [78, 79]. In this model, tmTNF expression by stromal cells appears critical, being required for peripheral arthritis and more pathogenic than tmTNF expressed by hematopoietic cells as for spondylitis. Moreover, as in the TNF $^{\Delta ARE}$  model, joint inflammation required the ubiquitous TNF receptor I, whereas TNF receptor II,

specifically expressed by hematopoietic cells, appeared to play a secondary role, notably in new bone formation.

#### *Implication of the IL-23/IL-17 cytokines axis*

IL-23 is a heterodimeric cytokine of the IL-12/IL-6 family, consisting of IL-23p19 and IL-12/23p40 subunits. Produced by APCs, it exerts an essential role in driving type 3 immune response involving Th17 cells and other sources of IL-17, including  $\gamma/\delta$  T cells, innate lymphoid cells 3, mucosal associated invariant T cells and invariant natural killer T cells (iNKT) as effector cells. Several animal models have implicated the IL-23/IL-17 signaling cascade in SpA, along with other experimental evidence, including numerous polymorphisms affecting genes within this axis that have been associated with SpA susceptibility, as mentioned above.

#### *a- IL-23 expression triggers SpA in mouse*

Sustained expression of IL-23 in mouse was achieved by the delivery of a minicircle DNA construct. This resulted in the development of enthesitis and enthesal new bone formation of the paws as well as the axial skeleton at the sites of attachment of the spinal ligaments to bone and the sacroiliac joint. Characteristic anatomic/clinical features of SpA also included psoriasis and inflammation in the aortic root and valve [80]. In this model, mouse entheses and aortic root were shown to contain a subset of resident IL-23 receptor-positive, retinoic acid receptor-related orphan nuclear receptor (ROR) $\gamma$ t+ CD4- CD8- T cells that belong mainly to the  $\gamma/\delta$  T cells population. These cells could respond to IL-23 by producing IL-22 and IL-17 that mediated enthesal inflammation and osteoproliferation [80, 81]. Silencing SOCS3, a negative regulator of cytokine/signal transducers and activators of transcription signaling potentiated IL-23-inducible SpA development [82]. The relevance of this model to the human context has been reinforced by the detection of comparable  $\gamma/\delta$  T cells residing in human entheses and the evidence for an enrichment of ROR $\gamma$ t+ iNKT and  $\gamma/\delta$  T cells exhibiting skewed Th17 phenotype in the joint of SpA patients [83, 84].

This model relies on an artificial source of IL-23, but other murine models have also highlighted the importance of the IL-23/IL-17 axis in more physiological situation. This is the case of the SKG strain variant of the BALB/c mouse which harbors a W163C mutation of ZAP-70, an adaptor protein signaling downstream of the T cell receptor, that results in

weakened signal. This mutation affects thymic selection, enriching the peripheral repertoire with IL-17-skewed autoreactive T cells and also altering the function of Foxp3<sup>+</sup> Tregs that are inefficient to suppress arthritis development [85]. Intraperitoneal injection of  $\beta$ -glucan-containing products such as curdlan in this mouse raised in specific pathogen-free conditions, was shown to induce a phenotype similar to SpA, consisting of dactylitis, Achilles tendon enthesitis, plantar fasciitis, destructive arthritis of ankles and wrists, spondylitis and sacroiliitis. Extra-articular features comprised uveitis and ileitis resembling CD [85]. The  $\beta$ -glucan is a molecular pattern that is present in plants, fungal cell walls and bacteria. It mimics a microbial stimulation by binding to the dectin-1 receptor that signals through CARD9 in APCs and activates the production of IL-12 and IL-23. The development of this inflammatory disease was shown to be dependent on IL-23 production in the ileum, acting through IL-17, and the production of those cytokines was dependent on microbiota because it was absent in germ-free mice [85]. Thus the production of IL-23 that can take place in the gut or even in the entheses appears as an essential cytokine in the development of SpA in some circumstances [86, 87].

*b- Murine model of SpA implicates IL-17 production independent of IL-23*

In patients, inhibition of IL-23 appeared not to be efficacious in all situations, with striking differences between PsA that responds well to anti-IL-23 treatment and axial SpA that does not [88, 89]. This observation differs from the broader success of IL-17 inhibitors in SpA which is consistent with experimental evidence in HLA-B27 transgenic rat and with the existence of IL-23-independent pathways of IL-17 production [90–92].

A model of SpA driven by IL-23-independent production of IL-17 was obtained by injecting intra-peritoneally in inbred mouse strains, mannan, a cell wall polysaccharide from the fungus *Saccharomyces cerevisiae*. It induced an acute inflammation resembling human PsA by combining hyperkeratinous skin predominantly on the ears and paws with arthritis in the articular joints, enthesitis and periostitis. In this model, adaptive immunity was not implicated. Mannan injection stimulated macrophages to produce TNF- $\alpha$ , which triggered  $\gamma\delta$  T cell activation and IL-17A production, which, in turn, were involved in recruiting neutrophils to the joints and skin, leading to disease phenotypes [93].

*c- The protective role of IL-27 in mouse model of SpA*



IL-27 belongs to the same family of cytokines as IL-12 and IL-23 [94]. It is active as a heterodimer consisting of the IL-27p28  $\alpha$ -subunit, coded by the *IL27A* gene, and the Epstein-Barr virus-induced protein 3  $\beta$ -subunit. Like IL-12 and IL-23, it is produced by APCs. It was initially reported to play a critical role in promotion of Th1 differentiation and IFN $\gamma$  production. However, subsequent studies revealed that IL-27 has broader stimulatory and inhibitory roles by inducing IL-10-producing Treg cells and opposing Th17 differentiation [40]. Interestingly, IL27A gene polymorphisms are associated with SpA predisposition and IL-27 production by DCs was altered in the HLA-B27 transgenic rat model, as described above. Genetic ablation of IL-27 receptor  $\alpha$  in mouse leads to axial bone changes reminiscent of AS by combining bone loss with ossification of the intervertebral disks. A small proportion of those mice developed neutrophilic dermatitis in the tail that progressed into the subcutaneous tissues and vertebral body of the tail, resulting in neutrophilic periostitis and osteitis [95]. This complex model also required a heterozygous deletion of P53 and was not associated with inflammation. However, it highlights the putative role of IL-27 to antagonize the bone consequences of SpA, i.e. osteoporosis and excessive ossification.

### **Spontaneous ankylosing enthesitis (ANKENT), a mouse model of PsA**

Aging male DBA/1 mice develop spontaneous dactylitis and nail disease, which sometimes develops into onychoperiostitis, reminiscent of PsA [96, 97]. Interestingly, occurrence of this ANKENT is increased by stress, a feature similar to SpA [98, 99]. By histology, the affected paws exhibit dactylitis and enthesitis with infiltration by polymorphonuclear cells and mononuclear cells in the subcutaneous tissue, tendon sheath and enthesis, as well as cartilage hyperplasia at the enthesal insertion followed by endochondral bone formation leading to joint space bridging.

This disease was shown as independent of T cells and TNF inhibition did not affect its development [96, 100]. In contrast, it was strongly inhibited by forcing the expression of *noggin*, a broad BMP antagonist. This therapeutic efficacy highlights the critical involvement of BMP signaling in the process, including the inflammatory changes, which may implicate Activin signaling antagonism as well as BMPs [101, 102].

### **Conclusions**

- Studies of the HLA-B27/hβ2m transgenic rat model of SpA have linked the pathogenicity of HLA-B27 to non-canonical properties of this molecule resulting in deregulated immune response, notably to stimuli of microbial origin.
- HLA-B27/hβ2m transgenic *Drosophila* unraveled a dominant negative interaction of HLA-B27 with BMPR1, which could account for increased signaling in response to BMP/TGFβ family ligands, leading to excessive inflammation and ossification.
- Several mouse models have highlighted the prominent roles of TNFα signaling and IL-23/IL-17 axis in the development of SpA manifestations.

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**Table 1.** Major characteristics of animal models used to study SpA mechanism.\*

Model	Phenotype	Triggering cells	Principle effector mechanism	Principal effector mediators	Major References
HLA-B27/h $\beta$ 2m transgenic rat	Arthritis, Psoriasis, UC, Epididymoorchitis or Arthritis, Spondylitis, Sacroliliitis, Eipdidymoorchitis	Dysfunctional HLA-B27+ APCs	Increased Th17 Deregulated Treg	<ul style="list-style-type: none"> <li>TNF<math>\alpha</math></li> <li>IL-17</li> </ul>	[22, 23, 26, 28, 30, 32, 45]
HLA-B27/h $\beta$ 2m transgenic <i>Drosophila</i>	Crossveinless Small eye	Stromal cells	Increased BMP/TGF $\beta$ signaling	<ul style="list-style-type: none"> <li>Activin</li> <li>BMP</li> </ul>	[63]
ERAP1 <sup>-/-</sup> mouse	Lumbar spine inflammation and ossification, Sacroillitis, Osteoporosis, Increased susceptibility to colitis	Decreased tolerogenic DC population and decreased expression of Qa-2 (the HLA-G murine ortholog) by tolerogenic DC and macrophages	Decreased CD3+CD4+Foxp3–IL 10+ inducible Treg population, possibly resulting from decreased Qa-2 expression	<ul style="list-style-type: none"> <li>Unknown</li> </ul>	[72]
TNF <sup>FAARE</sup> mouse	Polyarthritis, Enthesitis, Sacroiliitis, CD-like ileitis	Stromal cells (CD and Arthritis)  Myeloid and T cells (CD)	Stromal cells (Arthritis)	<ul style="list-style-type: none"> <li>TNF<math>\alpha</math> (Arthritis)</li> <li>IL-12 (CD)</li> <li>IFN<math>\gamma</math> (CD)</li> </ul>	[74–77]

			Th1 and CD8+ T cells (CD)		
tmTNF overexpressing mouse ( <i>TgA86</i> )	Arthritis, Enthesitis, Osteitis, Bony ankylosis (axial and peripheral joints)	Stromal cells ± haematopoietic cells	TNFR1 ± TNFR2 signaling	• tmTNF	[78, 79]
Minicircle IL-23 DNA-inducible SpA	Enthesitis, Enthesal ossification (paw, spine, sacroiliac joint), Psoriasis, Aortic root and valve inflammation	IL-23R+ RORγt+ CD4-CD8- γ/δT cells residing in entheses and aortic root	IL-23-dependent type 3 immune response	• IL-17 • IL-22	[80, 81]
Curdlan-induced SpA in SKG mouse (ZAP-70 mutation)	Arthritis, Enthesitis, Spondylitis, Sacroiliitis, Ileitis Uveitis	Curdlan-activated gut APCs ( <i>via</i> Dectin 1 receptor)	IL-23-dependent type 3 immune response	• IL-17	[85]
Fungus mannan inducible PsA	Arthritis, Enthesitis, Osteitis, Periostitis, Hyperkeratinous skin	TNFα-producing macrophages	γ/δ T cells-dependent type 3 immune response	• IL-17	[93]
IL27RA <sup>-/-</sup> P53 <sup>+/-</sup> mouse	Osteoporosis,	Unknown	Unknown	• Unknown	[95]

	Spine ossification, Neutrophilic dermatitis (rare), Osteitis and periostitis (rare)				
Ankylosing enthesitis (ANKENT) mouse	Dactylitis Onychoperiosteitis	Unknown	Increased BMP signaling	• BMP/TGF $\beta$ family ligands	[97, 101]

\* h $\beta$ 2m: human  $\beta$ 2-microglobulin; UC: ulcerative colitis; APC: antigen-presenting cell; Th17: T-helper 17 cell; Treg: regulatory T cell; TNF $\alpha$ : tumor necrosis factor- $\alpha$ ; IL-17: interleukin-17; BMP: bone morphogenic protein; TGF $\beta$ : transforming growth factor  $\beta$ ; DC: dendritic cell; CD: Crohn's disease; PsA: psoriatic arthritis; IL27RA: IL27 receptor  $\alpha$ .