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Characterization of Blood Mucosal-Associated Invariant T Cells in Patients With Axial Spondyloarthritis and of Resident Mucosal-Associated Invariant T Cells From the Axial Entheses of Non-Axial Spondyloarthritis Control Patients

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Objective. The importance of interleukin-17A (IL-17A) in the pathogenesis of axial spondyloarthritis (SpA) has been demonstrated by the success of IL-17A blockade. However, the nature of the cell populations that produce this important proinflammatory cytokine remains poorly defined. We undertook this study to characterize the major IL-17A–producing blood cell populations in the peripheral blood of patients with axial SpA, with a focus on mucosal-associated invariant T (MAIT) cells, a population known to be capable of producing IL-17.

Methods. We evaluated IL-17A production from 5 sorted peripheral blood cell populations, namely, MAIT cells, γδ T cells, CD4+ T cells, CD8+ T cells, and neutrophils, before and after stimulation with phorbol myristate acetate, the calcium ionophore A23187, and β-1,3-glucan. Expression of IL-17A transcripts and protein were determined using nCounter and ultra-sensitive Simoa technology, respectively. MAIT cells from the axial entheses of non-axial SpA control patients (n = 5) were further characterized using flow cytometric immunophenotyping and quantitative polymerase chain reaction, and the production of IL-17 was assessed following stimulation.

Results. On a per-cell basis, MAIT cells from peripheral blood produced the most IL-17A compared to CD4+ T cells ($P < 0.01$), CD8+ T cells ($P < 0.0001$), and γδ T cells ($P < 0.0001$). IL-17A was not produced by neutrophils. Gene expression analysis also revealed significantly higher expression of *IL17A* and *IL23R* in MAIT cells. Stimulation of peripheral blood MAIT cells with anti-CD3/CD28 and IL-7 and/or IL-18 induced strong expression of *IL17F*. MAIT cells were present in the normal, unaffected entheses of control patients who did not have axial SpA and showed elevated *AHR*, *JAK1*, *STAT4*, and *TGFB1* transcript expression with inducible IL-17A protein. IL-18 protein expression was evident in spinal enthesis digests.

Conclusion. Both peripheral blood MAIT cells and resident MAIT cells in normal axial entheses contribute to the production of IL-17 and may play important roles in the pathogenesis of axial SpA.

INTRODUCTION

Within the last 15 years, a clear role of the interleukin-23 (IL-23)/IL-17 axis underpinning the pathophysiology of axial spondyloarthritis (SpA) has emerged (1) as many of the genetic variants associated with ankylosing spondylitis (AS) susceptibility have been identified through genome-wide association studies. Among the genes found to be associated with AS are those in the IL-23/IL-

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17 pathway, including *IL23R*, *IL12B*, *IL6R, JAK2*, and *TYK2* (2). As IL-17 is the terminal cytokine of this pathophysiologic pathway, the development of new treatments initially focused on blocking this cytokine (3,4). In support of this hypothesis and beyond the findings of genetic association studies, an increased prevalence of IL-17–producing T helper (Th17) cells have been reported in the peripheral blood of axial SpA patients (5) and in the normal entheses of individuals who do not have axial SpA (6). Mucosal-associated invariant T (MAIT) cells and γδ T cells have been further described as alternative sources of IL-17A in the blood of patients with axial SpA (7,8). Appel et al also suggested that neutrophils might be the main producers of IL-17A in the facet joints of patients with AS (9).

IL-23 plays a crucial role in maintaining the differentiation state of Th17 cells. Nevertheless, recent studies have identified alternative pathways, independent of IL-23, that can stimulate IL-17 production, including T cell receptor (TCR) signaling through the engagement of major histocompatibility complex class I–related proteins by MAIT cells (10), IL-7 signaling in group 3 innate lymphoid cells (ILCs) (11), transforming growth factor β (TGFβ) and IL-1β signaling in invariant natural killer T (iNKT) cells (12,13) and γδ T cells (specifically the Vδ1 T cell subset) (14), and, recently, the combination of IL-12 and IL-18 together with anti-CD3/CD28 triggering in MAIT cells (10). Such observations have substantial translational relevance, given that antagonism of IL-23 has thus far shown no efficacy in the treatment of AS (15,16).

Considering that the growing body of evidence suggests a role for IL-17 in the pathogenesis of SpA, we wished to study the capability of MAIT cells, CD4+ T cells, CD8+ T cells, and γδ T cells to express IL-17 in patients with axial SpA. We first analyzed the cell type–specific expression patterns of AS-associated genes in those cells, with a particular focus on genes belonging to the IL-23/IL-17 pathway. We further compared the respective IL-17 production capacity of these different cell subsets from the adaptive and innate immune systems and identified MAIT cells as potent IL-17–secreting cells in patients with axial SpA. MAIT cells from healthy individuals have been reported to express high levels of IL-7 receptor (IL-7R) and IL-18R (17). In this study, we found that MAIT cells can produce IL-17 with combined TCR triggering and stimulation by IL-7 and IL-18, and independently of IL-23 stimulation. Additionally, we identified resident MAIT cells in the axial entheses of normal, healthy individuals, a site at which the unaffected tissue is targeted in the inflammatory process that leads to severe axial inflammation and later spinal fusion in patients with axial SpA. Taken together, our data highlight the crucial role of MAIT cells in the pathophysiology of axial SpA.

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PATIENTS AND METHODS

Patients and samples. *Axial SpA.* Blood samples from 18 patients naive to treatment with synthetic medications and biologics and with a clinical diagnosis of axial SpA who fulfilled the Assessment of SpondyloArthritis international Society criteria (18) were included in the study.

Enthesis tissue and peripheral blood samples from controls. As controls, human interspinous processes and matched peripheral blood samples were obtained from 5 non-axial SpA patients who underwent elective spinal surgery for either decompression or scoliosis correction using methods previously reported (19).

Before enrollment in the study, all patients provided written informed consent as approved by the French Ethics Committee and the North West–Greater Manchester West Research Ethics Committee. The clinical characteristics of the axial SpA patients and of the non-axial SpA control patients who underwent spinal surgery are summarized in Supplementary Table 1, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.42090.

Cell sorting and stimulation. The isolation of cell populations from peripheral blood samples and entheseal samples was undertaken as previously described (8,20) and is further described in the Supplementary Materials (http://onlinelibrary.wiley.com/doi/10.1002/art.42090).

Gene expression analysis. Gene expression profiles from the sorted cells of axial SpA patients were assessed using the nCounter Autoimmune Discovery panel (NanoString Technologies), and samples from the normal entheses of non-axial SpA patients were assessed using a focused gene card. Profiling of the transcription factors of entheseal and blood MAIT cells (CD3+, CD45+, CD161+, and TCRVα7.2+) was performed using the entheseal and peripheral blood samples from non-axial SpA patients. The basal transcript expression of cytokines, chemokines, growth factors, signaling molecules, and tissue residency markers were assessed using a focused gene card. A description of the RNA preparation and gene expression analysis is available in the Supplementary Materials (http://onlinelibrary.wiley.com/doi/10.1002/art.42090).

Protein expression analyses. The concentrations of IL-17A (expressed in fg/ml) in cell culture supernatants from axial SpA patients were determined using the Quanterix Simoa IL-17A Advantage Kit and HD-1 platform. Stimulated cells from

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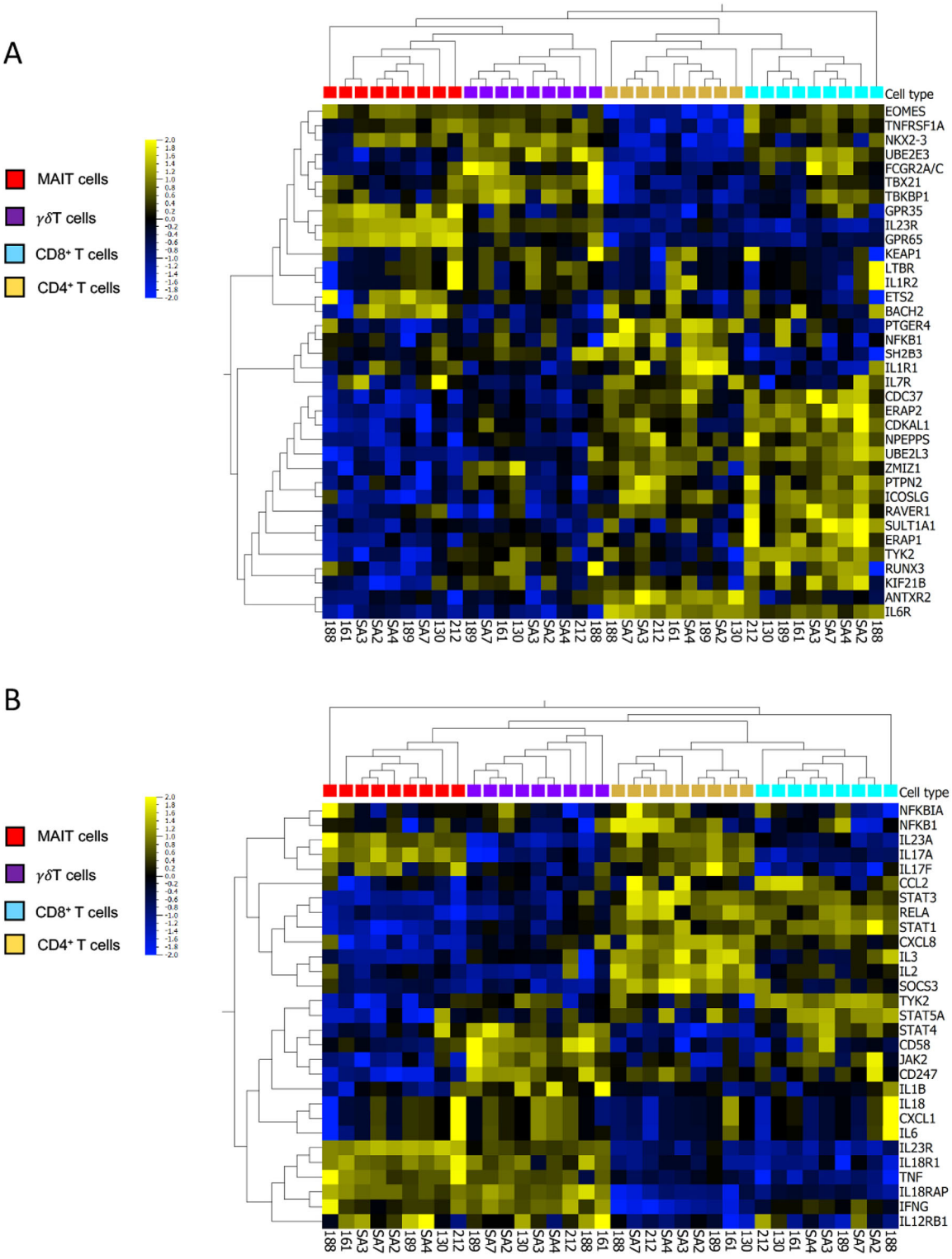

Figure 1. Heatmaps showing the expression patterns of genes associated with ankylosing spondylitis (AS) and of genes of the interleukin-23 (IL-23)/IL-17 pathway in T cell subpopulations isolated from the peripheral blood of patients with axial spondyloarthritis (SpA). **A**, Expression levels of 36 genes associated with AS susceptibility in T cells from axial SpA patients. **B**, Messenger RNA expression levels of 29 genes associated with the IL-23/IL-17 pathway, selected from the Molecular Signatures Database. T cells were stimulated for 2 hours with phorbol myristate acetate (50 ng/ml), calcium ionophore A23187 (5 μ*M*), and β-1,3-glucan (50 μg/ml). Heatmaps show hierarchical clustering of genes among T cell populations from individual patient samples (n = 9). Gene expression data are $\log_2$ transformed, centered to a mean value of 0, and scaled to unit variance. The color key on the left denotes the scale of gene expression, ranging from lower levels (blue) to higher levels (yellow).

perientheseal bone entheseal mononuclear cells (EMCs) were intracellularly stained for tumor necrosis factor (TNF) and IL-17 and analyzed using a flow cytometry gating system, as described previously (8,20). Following 24-hour stimulation with lipopolysaccharides (100 ng/ml), IL-18 protein was analyzed in the supernatant using BioLegend LEGENDplex Human Inflammation Panel 1 and the Beckman Coulter CytoFLEX LX Flow Cytometer, according to the manufacturer's instructions (Supplementary Figure 5, http://onlinelibrary.wiley.com/doi/10.1002/art.42090). Serum levels of IL-17A, IL-17F, IL-7, and IL-18 were quantified using the Olink Proximity Extension Assay (Supplementary Materials, http://onlinelibrary.wiley.com/doi/10.1002/art.42090).

Statistical analyses. GraphPad Prism software was used for statistical analyses. Detailed information regarding methods and statistical analyses is provided in the Supplementary Materials (http://onlinelibrary.wiley.com/doi/10.1002/art.42090).

RESULTS

Differential expression of genes associated with AS susceptibility in innate and adaptive T cell populations isolated from peripheral blood of axial SpA patients. Following stimulation with phorbol myristate acetate, the calcium ionophore A23187, and β-1,3-glucan, the expression of 36 genes from a panel of 45 genes associated with AS was analyzed in 4 T cell populations (MAIT cells, CD8+ T cells, CD4+ T cells, and γδ T cells) isolated from 9 axial SpA patients. The expression pattern observed after hierarchical clustering showed a clear distinction between the innate and adaptive T cell groups, as shown in the Figure 1A heatmap. Gene clusters consisting of members of the pathway network for major histocompatibility complex class I–mediated antigen processing and presentation (*NPEPPS* and *UBE2L3*) were up-regulated in CD4+ and CD8+ T cells. We observed that genes were expressed at relatively high levels in specific cell types, such as *PTGER4* in CD4+ T cells and *TYK2* in CD8+ T cells.

MAIT cells expressed high levels of *IL23R* and the G protein–coupled receptors *GPR35* and *GPR65*. We also noted cell type–specific expression of several IL-23/IL-17 pathway genes (Figure 1B). *IL17F* was expressed at higher levels (~1 log-fold difference in expression) in CD4+ T cells and MAIT cells compared to the other T cell populations, while *IL23R* expression was higher (2 log-fold difference) in MAIT cells and γδ T cell expression was higher (1 log-fold difference) than that in CD4+ and CD8+ T cells. *NFKB1*, *RELA*, and *NFKBIA* were preferentially expressed in CD4+ T cells, while several genes encoding cytokines and their receptors (*IL23A, IL23R, IL12RB1, IL18R1, IL18RAP, TNF*, and *IFNG*) were expressed at higher levels in innate-like MAIT cells and γδ T cells when compared to adaptive CD4+ and CD8+ T cells. *IL1R1, TYK2*, and *RUNX3* were expressed at high levels in CD8+ T cells. Nevertheless, many other genes not belonging to the IL-23/IL-17 pathway participated in cell clustering, suggesting that those different cell types were involved in AS susceptibility beyond their relative role in the IL-23/IL-17 pathway.

High potential for IL-17A and IL-17F secretion in peripheral blood–derived MAIT cells. To better define the relative role of MAIT cells, CD4+ T cells, CD8+ T cells, and γδ T cells in IL-17 expression, we further sorted these cell types together with neutrophils, whose secretion of IL-17A is still controversial (21). Cell sorting was performed on samples from

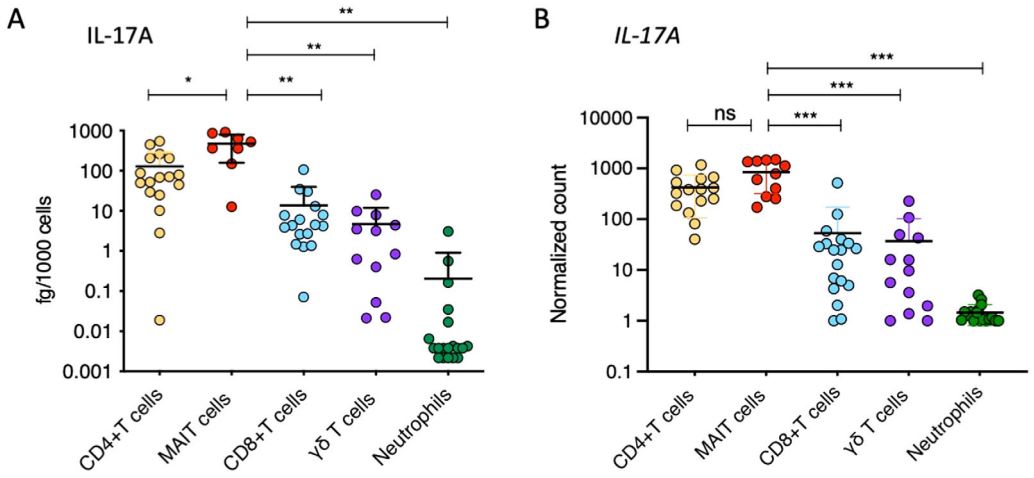

Figure 2. IL-17A protein production after 18 hours of stimulation (**A**) and *IL17A* transcript levels (normalized expression) after 2 hours of stimulation (**B**) were assessed in sorted CD4+ T cells, mucosal-associated invariant T (MAIT) cells, CD8+ T cells, γδ T cells, and neutrophils from the peripheral blood of axial SpA patients. Cells were stimulated with phorbol myristate acetate (50 ng/ml), calcium ionophore A23187 (5 μ*M*), and β-1,3-glucan (50 μg/ml). Symbols represent individual samples. Bars show the mean ± SEM. * = $P < 0.05$; ** = $P < 0.01$, by Mann-Whitney test. *** = $P < 0.001$, by Wilcoxon-Mann-Whitney test. ns = not significant (see Figure 1 for other definitions).

18 axial SpA patients (see Supplementary Figure 1 for the gating strategy, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42090). The production of IL-17A by MAIT cells was significantly higher than that of CD4+ T cells ($P < 0.05$), γδ T cells ($P < 0.01$), CD8+ T cells ($P < 0.01$), and neutrophils ($P < 0.01$). Although lower than in MAIT cells (mean 478.60 fg/1,000 cells), IL-17A production by CD4+ T cells was significant (mean 128.65 fg/1000 cells), while γδ T cells produced a smaller amount of IL-17A (mean 13.71 fg/1,000 cells), albeit in the same range as IL-17A production by CD8+ T cells (mean 4.66 fg/1,000 cells). The main component of *Aspergillus fumigatus* hyphae, β-glucan, has been demonstrated to have a potential effect on the production of IL-17A by human neutrophils (22). Despite strong β-glucan–associated stimulation, the expression of IL-17A by most neutrophils in the axial SpA patients did not exceed the detection limit (Figure 2A).

Gene expression analysis confirmed the findings of the IL-17A protein analysis, with MAIT cells and CD4+ T cells displaying the highest levels of *IL17A* expression (no significant difference between *IL17A* expression in MAIT cells and CD4+ T cells). A low

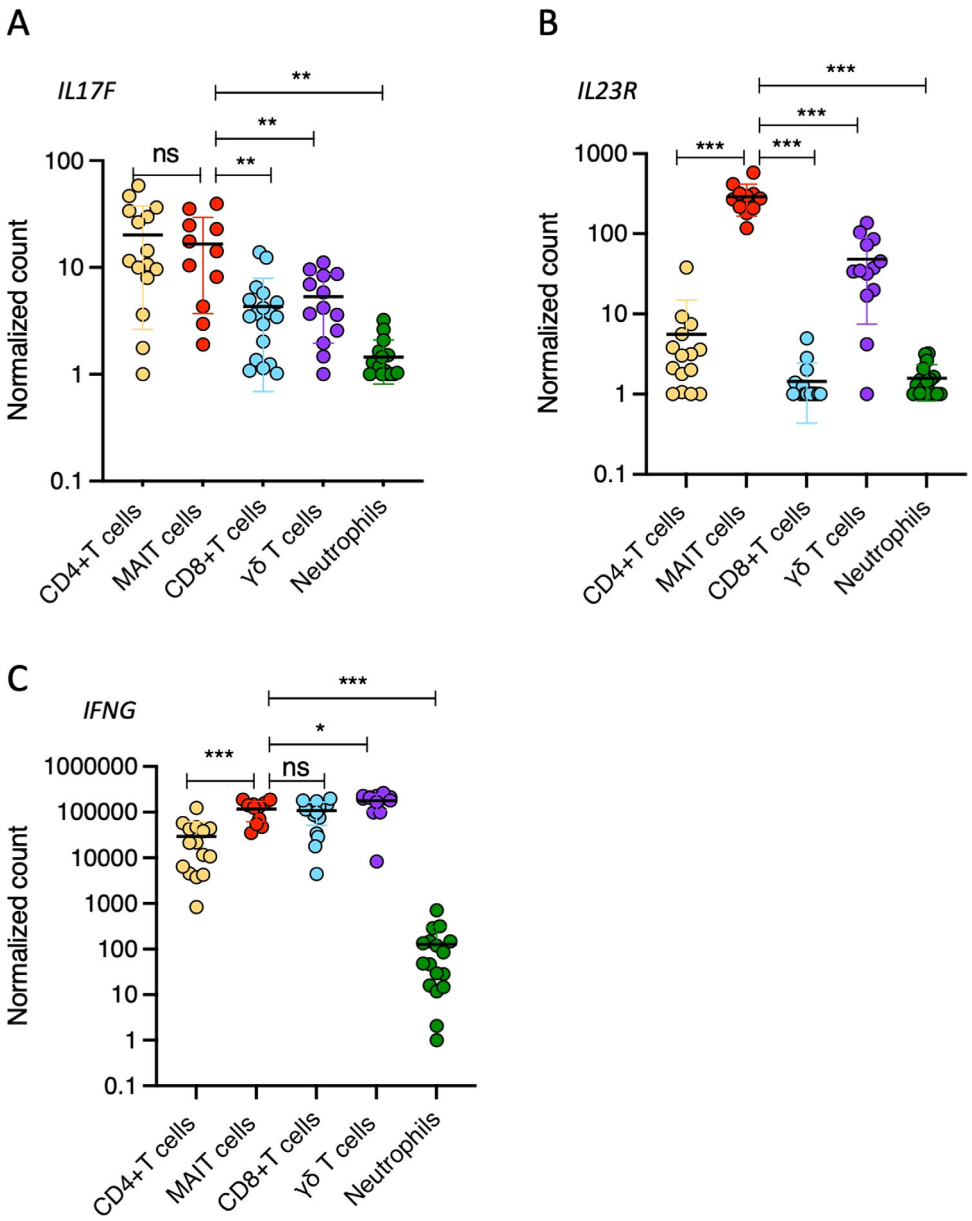

Figure 3. Sorted CD4+ T cells, MAIT cells, CD8+ T cells, γδ T cells, and neutrophils from the peripheral blood of axial SpA patients were analyzed for gene expression of *IL17F* (**A**), *IL23R* (**B**), and *IFNG* (**C**). Results are presented as normalized transcript levels. Cells were stimulated with phorbol myristate acetate (50 ng/ml), calcium ionophore A23187 (5 μ*M*), and β-1,3-glucan (50 μg/ml). Symbols represent individual samples. Bars show the mean ± SEM. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$, by Wilxocon-Mann-Whitney test. See Figure 2 for definitions.

level of *IL17A* expression was observed in γδ T cells and CD8+ T cells. In neutrophils, expression of *IL17A* was undetectable, which was consistent with the findings of the IL-17A protein analysis (Figure 2B).

Next, we expanded our analysis to include the expression of *IL17F*, *IL23R*, and *IFNG*. We observed that the 5 cell populations were similarly ranked in their expression levels of *IL17F* as they were in their expression levels of *IL17A* (Figure 3A), but there was no significant difference in the expression levels of *IL17F* between CD4+ T cells and MAIT cells. We also observed a 10-fold lower level of expression of *IL17F* compared to that of *IL17A* in all cell subsets. Regarding *IL23R* (Figure 3B), MAIT cells had the highest level of expression, followed by γδ T cells and CD4+ T cells, while CD8+ T cells and neutrophils did not express significant levels of *IL23R*. In contrast to *IL17A* and *IL17F* expression, *IFNG* was highly expressed in all T cell populations (Figure 3C), suggesting cell-specific expression profiles for *IL17A* and *IL17F*. Collectively, these data indicate that MAIT cells are the major producers of IL-17A on a per-cell level and express high levels of both *IL17F* and *IL23R* compared to other IL-17A–producing cells in patients with axial SpA.

Enhancement of *IL17F* expression by IL-7 and IL-18 in peripheral blood–derived MAIT cells. We further assessed which stimulation conditions induced IL-17A and IL-17F protein expression by MAIT cells. Sorted MAIT cells (see Supplementary Figure 2 for the gating strategy, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42090) were stimulated for 36 hours with anti-CD3/anti-CD28, anti-CD3/anti-CD28 in combination with either IL-7 or IL-18, and anti-CD3/anti-CD28 combined with both IL-7 and IL-18. We used CD4+CCR6+ T cells ("Th17-like" T cells) as controls (Figure 4). We observed that stimulation with anti-CD3/anti-CD28 combined with IL-7 or IL-18 induced high expression levels of *IL17F* but not *IL17A* in MAIT cells (Figures 4A and B). Furthermore, we identified increased *IL18R1* expression (Figure 1B) by MAIT cells, which supports the finding that IL-18 induces *IL17F* production. The combination of both IL-7 and IL-18 with anti-CD3/anti-CD28 further increased *IL17F* expression. Expression levels of *IFNG* were also remarkably high after MAIT cells were stimulated with anti-CD3/anti-CD28 and either IL-7 or IL-18 and when they were stimulated with anti-CD3/anti-CD28 and both IL-7 and IL-18 combined (Figure 4C).

Basal levels of IL-18 expression were demonstrated in MAIT cells isolated from the normal entheses of patients who did not

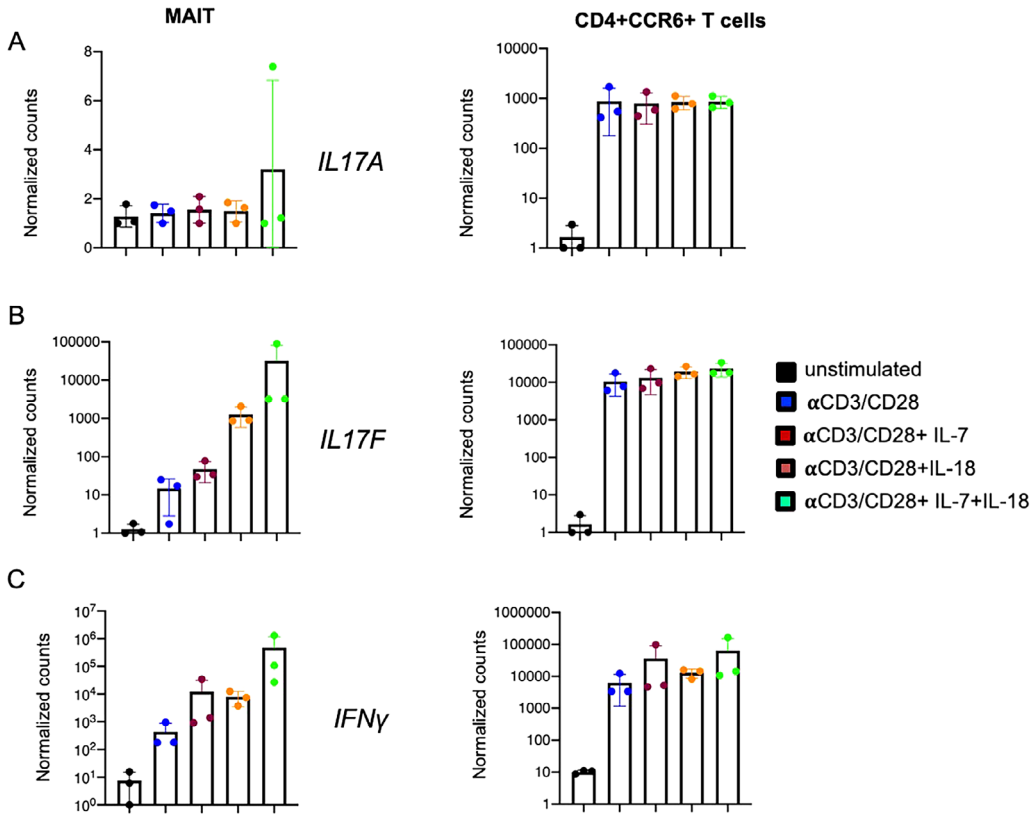

Figure 4. Gene expression of *IL17A* (**A**), *IL17F* (**B**), and *IFNG* (**C**) in sorted mucosal-associated invariant T (MAIT) cells and CD4+CCR6+ T cells from non-axial SpA patients (n = 3) after 36 hours of stimulation in 6 different conditions, including unstimulated, stimulation with anti-CD3/anti-CD28, stimulation with anti-CD3/anti-CD28 and interleukin-7 (IL-7) (20 ng/ml), stimulation with anti-CD3/anti-CD28 and IL-18 (50 ng/ml), and stimulation with anti-CD3/anti-CD28 and both IL-7 (20 ng/ml) and IL-18 (50 ng/ml). Results are presented as the mean ± SEM normalized transcript levels. Symbols represent individual samples.

have axial SpA (Supplementary Figure 5, http://onlinelibrary.wiley.com/doi/10.1002/art.42090), which arguably supports the importance of IL-18 in MAIT cell modulation. In peripheral blood samples, the comparison between axial SpA patients and non-axial SpA control patients did not reveal a significant difference in the serum production of IL-17A and IL-18, but a trend toward increased expression of *IL17F* and *IL7* in axial SpA patients was observed (P = 0.0508 and P = 0.06, respectively) (Supplementary Figure 6, http://onlinelibrary.wiley.com/doi/10.1002/art.42090). In MAIT cells, no significant difference in IL-17A secretion or *IL17A* gene expression was observed (Supplementary Figures 7 and 8, http://onlinelibrary.wiley.com/doi/10.1002/art.42090).

Presence of MAIT cells in entheses unaffected by axial SpA. Considering that the hallmark of axial SpA pathogenesis is entheseal inflammation, we investigated and confirmed the presence of MAIT cells in the axial entheses of individuals who did not have axial SpA (Figure 5A). Within both entheseal soft tissue and perientheseal bone, MAIT cells mainly expressed the resident memory marker CD69, while MAIT cells from the peripheral blood mainly expressed CD45RA, which corresponds to a naive/circulating phenotype (Figure 5A).

Transcriptional profiling of entheseal MAIT cells compared to peripheral blood–derived MAIT cells. The comparison of peripheral blood–derived and enthesis-derived (from both perientheseal bone and entheseal soft tissue) MAIT cell transcription factors (TCRVα7.2+ and CD161+) showed that entheseal soft tissue MAIT cells had a phenotype characterized by higher *AHR, JAK1*, *STAT4*, and *TGFβ1* transcript expression

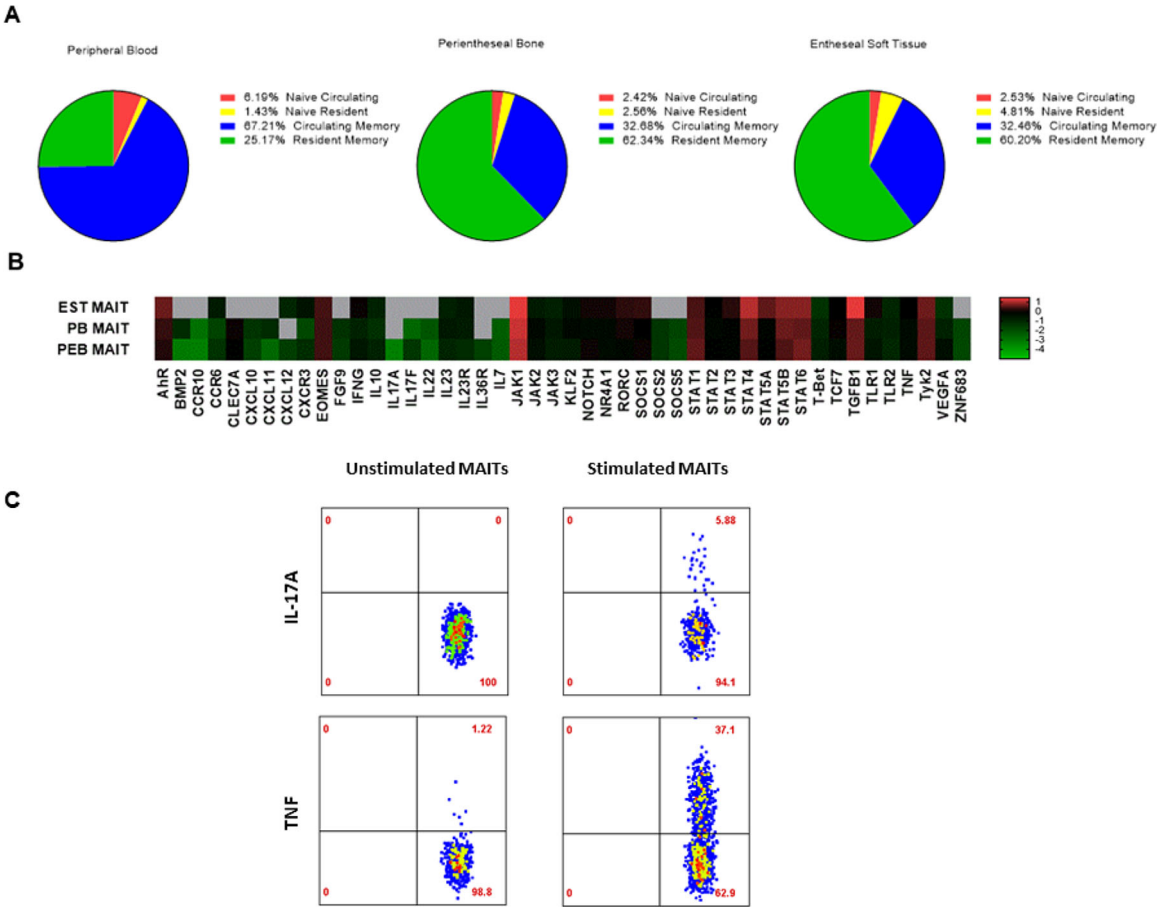

Figure 5. Transcriptional profiling of entheseal mucosal-associated invariant T (MAIT) cells and proinflammatory cytokine induction. **A**, Stratification of MAIT cell subsets based on their expression of T cell receptor Vα7.2 and CD161 in entheseal soft tissue (EST), perientheseal bone (PEB), and peripheral blood (PB) from non-axial spondyloarthritis patients. MAIT cells expressing tissue resident/memory markers were identified by CD69 expression, and naive/circulating MAIT cells were identified by CD45RA expression. Results are shown as the mean percentage (n = 5). **B**, Basal expression of cytokines, chemokines, growth factors, signaling molecules, and tissue residency markers. Expression values are the $\log_{10}$ change in threshold cycle relative to the values for hypoxanthine guanine phosphoribosyltransferase (n = 7). The color key denotes differential gene expression, in which values <–1 indicate lower relative expression and values >1 indicate higher relative expression. Gray indicates absence of expression. P = 0.038 by 2-tailed t-test for independent samples for the difference in expression of *CCR6* by MAIT cells from peripheral blood and MAIT cells from perientheseal bone. **C**, Intracellular tumor necrosis factor (TNF) and interleukin-17 (IL-17) cytokine expression in conditions with or without stimulation with phorbol myristate acetate (50 ng/ml) and ionomycin (1 μg/ml) for 3 hours in the presence of BD GolgiPlug protein transport inhibitor in perientheseal bone–derived MAIT cells (n = 2).

(Figure 5B). Furthermore, transcription factors indicative of circulating T cells, such as *KLF2* and *TBX21*, showed higher expression in peripheral blood–derived MAIT cells (Figure 5B). Enthesis-derived MAIT cells also showed higher expression of growth factors and molecules associated with tissue repair and homeostasis, such as *VEGFA* and *IL10*, when compared to matched peripheral blood–derived MAIT cells (Figure 5B).

In comparison to blood-derived MAIT cells, enthesis-derived MAIT cells showed higher *CXCR3* and *CCR6* expression. These findings suggest that enthesis-derived MAIT cells are better equipped to mediate proinflammatory signals and tissue migration.

Induction of TNF and IL-17 expression in entheseal MAIT cells. Following on previous findings that blood-derived MAIT cells produced IL-17, we next investigated whether entheseal MAIT cells also had the ability to produce this cytokine. Following stimulation with phorbol myristate acetate and ionomycin, MAIT cells showed elevated expression of TNF and IL-17A through intracellular flow (Figure 5C). Overall, 3.2% of stimulated MAIT cells expressed IL-17A and 17.9% of stimulated MAIT cells expressed TNF (n = 2 independent samples with MAIT cells). Given that IL-18 was shown to enhance IL-17 production, we also investigated the expression of IL-18. Our results confirmed that IL-18 was expressed at basal levels in the normal entheses (Supplementary Figure 5, http://onlinelibrary.wiley.com/doi/10.1002/art.42090).

DISCUSSION

The findings presented herein suggest a potentially important role for MAIT cells in the setting of axial SpA. Gene expression profiles based on gene polymorphisms associated with AS showed substantial clustering in MAIT cells. Furthermore, MAIT cells had at least as much IL-17A production capacity as CD4+ T cells. In addition, MAIT cells were able to produce *IL17A* and *IL17F* under conditions of combined stimulation with IL-7 and IL-18 together with TCR triggering. Finally, MAIT cells were present in the spinal entheses of healthy controls, where they were mainly of the resident memory cell phenotype with inducible IL-17A protein production. Collectively, these findings substantially add to the body of evidence incriminating innate-like lymphocytes in the pathogenesis of axial SpA.

We first analyzed the gene expression profiles of 45 genes whose polymorphisms were significantly associated with the predisposition to AS in 4 T cell populations: CD4+ T cells, CD8+ T cells, γδ T cells, and MAIT cells. Eighty percent of these genes were expressed among these 4 cell types, with differential expression from one cell population to another leading to cell subset clustering. We observed higher expression levels of prostaglandin receptor EP_4 (*PTGER4*) in CD4+ T cells, through which prostaglandin E_2 regulates Th17 cell differentiation and functioning (23). This up-regulation of the receptor EP_4 in axial SpA patients could promote CD4 differentiation toward the Th17 pathway, as has been previously reported (23). *TYK2* was expressed at higher levels in adaptive CD8+ T cells, followed by innate-like γδ T cells. Tyk2 is a crucial type 3 immunity mediator in SpA, and its inhibition can prevent disease progression by reducing the expansion of Th17 cells in murine models of SpA (24). Inhibition of Tyk2 also showed promising results in a phase II trial in psoriasis (25).

We observed an up-regulation of *IL18R1* and *IL18RAP* in MAIT cells, in accordance with the sensitivity of MAIT cells to IL-18 stimulation for the induction of IL-17. High expression of IL-18R and IL-12R by MAIT cells has been shown to facilitate their activation in a TCR-independent manner, such as during viral infections (26). The relatively high expression of the AS-associated G protein–coupled receptor genes *GPR35* and *GPR65* in MAIT cells could indicate their pathogenic role in the setting of axial SpA, as increased expression of *GPR65* in granulocyte–macrophage colony-stimulating factor–positive CD4+ T cells has been associated with "pathogenic" Th17 cells in SpA patients (27). These results could help us to design functional analyses specifically for MAIT cells.

To better decipher the relative contribution of MAIT cells to IL-17A/IL-17F expression in comparison to CD4+ T cells, we assessed the secretion of IL-17A in cell culture supernatants after stimulation. Generally, MAIT cell frequency is relatively low compared to CD4+ T cells and CD8+ T cells. However, on a per-cell basis, MAIT cells were the major producers of IL-17A in axial SpA patients when compared to CD4+ T cells, CD8+ T cells, and γδ T cells. Analysis on a per-cell basis allowed us to precisely characterize the production capacity of each cell type, which is generally challenging for small cell subsets.

MAIT cells strongly expressed *IL23R*, but our work shows that cytokines other than IL-23, such as IL-7/IL-18 alone or in combination, can induce strong expression of *IL17F* messenger RNA. We found particularly high levels of *IL18R1* and *IL18RAP* expression in MAIT cells, suggesting that IL-18 is an important cytokine in the modulation of their function. Furthermore, we found IL-18 production at the basal level in unstimulated MAIT cells derived from the perientheseal bone of non-axial SpA patients, which demonstrates the importance of IL-18 in MAIT cell regulation. IL-18, which is associated with IL-12 and IL-15, induces IFNγ secretion by Th1 cells (28) and has been shown to synergize with IL-12 to promote the production of IL-17A/IL-17F by MAIT cells independently of IL-23 (10).

Here we show that another cytokine combination (IL-7 and IL-18) particularly potentiates the production of IL-17F by MAIT cells. To our knowledge, this is the first time that a combination of cytokines has been shown to induce the expression of *IL17F* by MAIT cells in the setting of axial SpA. IL-7 is a key cytokine of the adaptive immune system not only for the development of T cells and dendritic cells, but also for the expansion and survival of immature B cells. While stromal and epithelial cells are known

to be the main producers of IL-7, a study by Ciccia et al demonstrated that intestinal Paneth cells could also produce IL-7 (29). IL-7 is constitutively produced at low levels, but elevated levels of IL-7 have been observed in the sacroiliac joint fluid of patients with SpA (30). Recently, IL-17F has gained interest with the approval of bimekizumab for the treatment of psoriasis (31), and bimekizumab has also shown promising results in patients with axial SpA (32). In the present study, we did not focus on the role of *IL23R* in the pathogenesis of axial SpA. Additional studies with specific approaches to better deciphering the complexity of the interactions in the different IL-17–producing cells are needed.

In this work, the contribution of γδ T cells and CD8+ T cells from peripheral blood to the production of IL-17A was minimal, but it is possible that tissue-specific expression of IL-17A is much higher at sites targeted during the inflammatory process in axial SpA. While neutrophils have previously been described as IL-17–producing cells (22), our work with 2 robust techniques (nCounter and Simoa technology) did not confirm this, even when strong stimulation combining phorbol myristate acetate, the calcium ionophore A23187, and β-1,3-glucan was used. This supports the previous finding that neutrophils do not substantially contribute to the production of IL-17 (21).

Several reports have suggested that in the peripheral form of axial SpA, MAIT cells are not the only cells which produce IL-17. Both iNKT and γδ T cells are increased in the synovial fluid of patients with SpA and contribute to IL-17 expression, but they contribute through an IL-23–dependent mechanism (13). This may be in part due to the shared transcription factor promyelocytic leukaemia zinc-finger (PLZF; encoded by *ZBTB16*), which is present in MAIT cells, iNKTs, and γδ T cells (33). Kenna et al observed high expression of IL-23R on the surface of γδ T cells (6), but this cell population did not appear to be the main source of IL-17 production in the axial form of SpA. Group 3 innate lymphoid cells are also capable of producing IL-17A, but Blijdorp et al (34) recently demonstrated that these cells produced IL-22 rather than IL-17A in the joints of patients with peripheral SpA.

This study had some limitations which can be addressed in future research, such as the assessment of local production of IL-7 and IL-18 within the entheseal tissues of axial SpA patients. It would be ideal to use matched MAIT cells from the blood and entheses of patients with axial SpA, but entheseal tissue samples from axial SpA patients are difficult to access.

In this study, we used data from genome-wide association studies to examine transcript expression in cells relevant to the pathogenesis of axial SpA, and given the recent and unexpected developments in translational therapeutics for the treatment of SpA where IL-17 inhibition rather than IL-23 inhibition was effective, we focused our research on the IL-23/IL-17 pathway. Our findings support the hypothesis of major involvement of conventional T cell subsets and innate-like lymphocytes in the pathogenesis of SpA. However, MAIT cells from peripheral blood were shown to be the main producers of IL-17A in axial SpA patients when compared to CD4+ T cells, CD8+ T cells, and γδ T cells. Moreover, the stimulation of MAIT cells with combined IL-7 and IL-18 was able to strongly induce *IL-17F* expression. A trend toward increased expression of IL-17F and IL-7 was also observed in the serum of axial SpA patients compared to control patients. The importance of MAIT cells was highlighted by their identification within entheseal tissues where basal IL-18 protein expression was also found. Further studies are needed to assess whether similar findings hold true in the entheseal tissues from axial SpA patients and to what extent the in situ production of IL-7 and IL-18 induces IL-17F expression.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Miceli-Richard had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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