The role of innate immune cells in tissue inflammation in spondyloarthritis
Noordenbos, T.

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INTERLEUKIN-17–POSITIVE MAST CELLS CONTRIBUTE TO SYNOVIAL INFLAMMATION IN SPONDYLARTHRITIS

Troy Noordenbos¹, Nataliya Yeremenko¹, Ioana Gofita¹, Marleen van de Sande¹, Paul P. Tak¹, Juan D. Cañete², and Dominique Baeten¹

¹Academic Medical Center/University of Amsterdam, Amsterdam, The Netherlands,
²Hospital Clinic de Barcelona and Institut d’Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain

Drs. Noordenbos and Yeremenko contributed equally to this work

ABSTRACT

Objective
Studies comparing spondylarthritis (SpA) to rheumatoid arthritis (RA) synovitis suggest that innate immune cells may play a predominant role in the pathogenesis of SpA. Recent observations have indicated a marked synovial mast cell infiltration in psoriatic SpA. We therefore undertook the present study to investigate the potential contribution of mast cells to synovial inflammation in SpA.

Methods
Synovial tissue and fluid were obtained from patients with either nonpsoriatic or psoriatic SpA (n = 82) and patients with RA (n = 50). Synovial biopsy tissue was analyzed by immunostaining and used in ex vivo cultures. Synovial fluid was analyzed by enzyme-linked immunosorbent assay.

Results
We observed a strong and specific increase of c-Kit–positive mast cells in the synovium from patients with SpA compared to the synovium from patients with RA synovitis, which was independent of disease subtype (nonpsoriatic versus psoriatic), disease duration, and treatment. Staining of mast cell granules, analysis of synovial fluid, and results in ex vivo tissue culture did not indicate increased degranulation in SpA synovitis. However, mast cells expressed significantly more interleukin-17 (IL-17) in SpA than in RA synovitis, and mast cells constituted the major IL-17–expressing cell population in the SpA synovium. Ex vivo targeting of synovial mast cells with the c-Kit inhibitor imatinib mesylate significantly decreased the production of IL-17 as well as other proinflammatory cytokines in synovial tissue cultures. Analysis of paired pre- and posttreatment synovial tissue samples indicated that the mast cell/IL-17 axis in SpA was not modulated by effective tumor necrosis factor (TNF) blockade.

Conclusion
The specific and TNF-independent increase in IL-17–expressing mast cells may contribute to the progression of synovial inflammation in peripheral SpA.
Spondylarthritis (SpA) and rheumatoid arthritis (RA) are the two most frequent forms of chronic immune-mediated inflammatory arthritis. Although both diseases affect the peripheral joints, there are striking clinical differences in sex distribution, age at disease onset, and pattern of joint involvement. The cellular and molecular pathways responsible for the differences and commonalities between these two types of arthritis remain largely unknown. Based on a series of systematic synovial studies, we recently proposed that joint inflammation in SpA may be driven mainly by cells of the innate immune system, including specific macrophage subsets and granulocytes, whereas molecular pathways related to [auto]antigen-specific T and B lymphocyte activation predominate in RA synovitis [1–10].

An important cellular component of the innate immune system is the mast cell. Mast cells are important sentinel cells of the skin and the gut mucosa, but are also present in other tissues, including the synovial membrane. They can get activated by cross-linking of Fc receptors with immunoglobulins, as well as by complement (C5a and C3), Toll-like receptor ligands, and stem cell factor (SCF). Upon activation, they produce and/or secrete a wide array of mediators that contribute to immune defense and inflammation. This includes not only proteases and histamine but also prostaglandins and leukotrienes, growth factors such as platelet-derived growth factor (PDGF), basic fibroblast growth factor, and vascular endothelial growth factor (VEGF), and cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1) [11]. These mediators can drive, amplify, and perpetuate a variety of aspects of tissue inflammation: induction of vascular permeability, endothelial activation with expression of adhesion molecules, recruitment of circulating leukocytes, and activation of resident stromal cells.

Based on their inflammatory potential and their ~10-fold increase in rheumatoid synovial tissue as compared to normal synovial tissue, mast cells have been proposed to play an important role in synovial inflammation in RA [12–14]. This concept is supported by findings in experimental arthritis models, as well as by data in human RA, including the demonstration that synovial mast cells are important producers of TNF, IL-1β, and IL-17 [15–18]. In contrast, the presence and function of mast cells have not been assessed in SpA. In a recent study on the synovial immunopathologic processes of Behçet’s disease, we observed a marked synovial infiltration with mast cells in patients with psoriatic arthritis (PsA), one of the prevalent phenotypic subtypes of SpA [19]. Considering the potential role of aberrant innate immune responses in the pathogenesis of SpA [9,10], this observation urged us to investigate the presence and potential role of mast cells in SpA synovitis.

PATIENTS AND METHODS
 Patients and tissue samples
 Samples of synovial tissue and fluid were obtained from patients with SpA (n = 82) whose diagnosis fulfilled the European Spondylarthropathy Study Group criteria [20]. In order to assess potential differences between SpA subtypes, these patients were further subdivided into those with nonpsoriatic SpA (consisting of ankylosing spondylitis and undifferentiated SpA) and those with the psoriatic form, PsA, with the latter group fulfilling the Classification of Psoriatic Arthritis Study Group Criteria [21]. Patients with RA [n = 50] whose diagnosis
fulfilled the American College of Rheumatology classification criteria for RA [22] were included as an inflammatory disease control.

The total study population consisted of 4 cohorts. In the first 3 cohorts, synovial tissue biopsy samples were obtained from the inflamed knee joints by needle arthroscopy, as described previously [23]. Cohort 1 consisted of 16 patients with nonpsoriatic SpA, 23 with PsA, and 21 with RA. Cohort 2 consisted of 11 patients with SpA and 16 with RA, all of whom had early (disease duration <1 year), untreated disease. Cohort 3 consisted of 11 patients with SpA, from whom paired synovial biopsy tissue samples were obtained before and 12 weeks after TNF blockade with etanercept. Finally, for cohort 4, synovial fluid samples were obtained by arthrocentesis from the joints of 21 patients with SpA and 13 patients with RA. The demographic and clinical characteristics of the 4 cohorts are shown in Table 1. All patients gave their written informed consent to participate to the study, as approved by the Medical Ethics Committee of the Academic Medical Center/University of Amsterdam.

Immunohistochemistry

Synovial biopsy samples (6–8 per patient) were snap-frozen and mounted in Jung tissue-freezing medium (Leica Instruments). Frozen sections were fixed and stained with monoclonal antibodies directed toward CD3 (clone UHT1; Dako), CD20 (clone L26; Dako), CD34 (clone 4H11; BioLegend), CD68 (clone EBM-11; Dako), CD117 (c-Kit, clone 104D2; BioLegend), CD127 (clone HCD127; BioLegend), CD163 (clone 5cFAT; BMA Biomedicals), and anti–mast cell tryptase (clone AA1; Abcam). After extensive rinsing, sections were sequentially incubated with a biotinylated secondary antibody, a streptavidin–horseradish peroxidase link (LSAB; Dako), aminoethylcarbazole substrate as chromogen, and hematoxylin as counterstain. Parallel sections were incubated with irrelevant isotype- and concentrationmatched monoclonal antibodies as negative control. Additional sections were stained with toluidine blue (Sigma-Aldrich) rather than hematoxylin, to stain mast cell granules.

In specific experiments, a single section was stained with toluidine blue in order to localize mast cell granules, and the tissue sections were subsequently washed and stained with anti–c-Kit in order to colocalize the mast cell granules and c-Kit, determined by consecutive staining. For each cohort, all samples were coded and stained in a single run in order to minimize technical biases. Stained sections were subsequently scored, in a random order, for cellular infiltration on a 4-point semiquantitative scale, as described previously [2–4]. Scoring was carried out by two independent observers (TN and NY) who were blinded to each patient’s diagnosis and clinical data.

Double immunofluorescence

Five-micrometer sections of synovial tissue from patients with SpA and patients with RA were deparaffinized, rehydrated, and incubated with 0.5% hydrogen peroxidase/methanol. After antigen retrieval by heating in 0.5M citrate buffer (pH 6), the sections were blocked with 10% rabbit serum. Stainings with mouse anti–mast cell tryptase (clone AA1; Abcam), anti-CD15 (clone C3D-1; Dako), or anti-CD3 (clone LN10; Vector Laboratories) and goat anti–IL-17 (R&D Systems) were performed overnight at 4°C, followed by incubation with Alexa Fluor 488/
Table 1. Demographic and clinical features of the patients in the 4 cohorts

<table>
<thead>
<tr>
<th></th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
<th>Cohort 4</th>
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<tr>
<td></td>
<td>SpA (n = 16)</td>
<td>PsA (n = 23)</td>
<td>RA (n = 21)</td>
<td>SpA (n = 11)</td>
</tr>
<tr>
<td>Age, years</td>
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<td>46 (24-66)</td>
<td>54 (21-70)</td>
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<tr>
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<td>12/11</td>
<td>6/15</td>
<td>8/3</td>
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<td>Disease duration, years</td>
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<td>7 (1-25)</td>
<td>7 (1-32)</td>
<td>0.6 (0.1-0.9)</td>
</tr>
<tr>
<td>Swollen joint count</td>
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<td>2 (1-17)</td>
<td>8 (1-16)</td>
<td>2 (1-3)</td>
</tr>
<tr>
<td>CRP, mg/liter</td>
<td>15 (5-86)</td>
<td>28 (5-124)</td>
<td>21 (1-185)</td>
<td>21 (2-60)</td>
</tr>
<tr>
<td>ESR, mm/hour</td>
<td>16 (3-64)</td>
<td>15 (6-58)</td>
<td>28 (2-87)</td>
<td>16 (5-35)</td>
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<tr>
<td>No. taking DMARDs</td>
<td>8</td>
<td>16</td>
<td>15</td>
<td>0</td>
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</tbody>
</table>

* Except where indicated otherwise, values are the median (interquartile range). In cohort 1, synovial biopsy tissue was obtained from patients with nonpsoriatic spondylarthritis (SpA), patients with psoriatic arthritis (PsA), and patients with rheumatoid arthritis (RA). In cohort 2, synovial tissue was obtained from patients with SpA and patients with RA whose disease was early (duration <1 year) and untreated. In cohort 3, paired synovial tissue biopsy samples were obtained from patients with SpA before (baseline) and 12 weeks after tumor necrosis factor blockade with etanercept. In cohort 4, synovial fluid samples were obtained from patients with SpA and patients with RA. CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; DMARDs = disease-modifying antirheumatic drugs.
Alexa Fluor 555–conjugated goat anti-mouse and goat anti-rabbit secondary antibodies. Slides were mounted with Vectashield containing DAPI (Vector Laboratories) and analyzed on a fluorescence imaging microscope (Leica DMRA) coupled to a CCD camera, with results analyzed using Image-Pro Plus software (Media Cybernetics, Dutch Vision Components). Coexpression of mast cell tryptase with IL-17 was determined by the manual counting of positive cells in 10 high-power fields, performed by two independent, blinded observers (NY and IG).

**Ex vivo biopsy cultures**

Fresh synovial tissue biopsy samples (at least 8 per patient) obtained from the patients’ clinically inflamed knee joints were pooled, divided in 2 equal fractions, weighed, and put into culture in 1 ml of Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, L-glutamine, HEPES, and antibiotics (penicillin/ gentamicin/streptomycin) (all from Gibco). One fraction was cultured in medium alone, whereas the paired second fraction was cultured in medium complemented with 2 μM of imatinib mesylate (Novartis). The culture medium and synovial biopsy tissue samples were recovered after 4 days of culture for enzyme-linked immunosorbent assay (ELISA) analysis and messenger RNA (mRNA) extraction.

**ELISA**

The levels of soluble molecules in synovial fluid or in culture medium were determined using specific ELISAs in accordance with the manufacturers’ instructions. ELISAs were performed for tryptase (Phadia ImmunoCAP), histamine (Neogen), SCF (R&D Systems), soluble ST2 (Enzo Life Sciences), IL-6 (Sanquin) (all with a detection limit of 0.2 pg/ml), IL-8 (detection limit of 1 pg/ml; Sanquin), and IL-17A (detection limit of 3 pg/ml; Abcam).

**Real-time quantitative polymerase chain reaction (qPCR)**

Messenger RNA was extracted from the synovial biopsy tissue after 4 days of in vitro culture, using RNA Stat-60 (Tel-Test), and then treated with DNase I (Invitrogen) and reverse-transcribed using a RevertAid H Minus First Strand complementary DNA Synthesis Kit (Fermentas). The RNA concentration was determined with a NanoDrop assay (NanoDrop Technologies). Analysis of mRNA by qPCR was performed using a StepOnePlus Real-Time PCR System (Applied Biosystems). Predesigned TaqMan probe and primer sets for TNF (Hs00174128_m1), IL-6 (Hs00174131_m1), IL-8 (Hs00174103_m1), IL-17A (Hs00174383_m1), and GAPDH (4310884E) were chosen from an online catalog (Applied Biosystems) and assayed according to the manufacturer’s protocol.

**Statistical analysis**

Because the data were not normally distributed, they were expressed as the median (interquartile range (IQR)) and were analyzed with nonparametric statistical tests: the Mann-Whitney U test for comparison of groups, and the Spearman’s rank test for correlations. *P* values less than 0.05 were considered statistically significant.
RESULTS
Detection of mast cells in SpA synovitis

In line with our previous observations [19], staining with anti-CD117, which was used to identify c-Kit–positive cells, in the inflamed synovial tissue from patients with nonpsoriatic SpA and patients with PsA revealed the presence of a clear population of large mononuclear cells in the synovial sublining (Figure 1A). Of note, c-Kit is commonly used as a phenotypic marker for mast cells, but can also be expressed by other cell populations, such as CD34-positive hematopoietic stem cells and CD127-positive lymphoid inducer cells. In the SpA synovial tissue, double staining with anti-CD34 and anti-CD127 did not reveal coexpression with c-Kit, whereas a clear colocalization of anti-CD117 and toluidine blue was observed in consecutive stainings of single sections, thus identifying the presence of mast cell granules (Figures 1B and C). In addition, there was colocalization of c-Kit and mast cell tryptase, as revealed by double immunofluorescence (Figures 1D–F). These findings confirm the presence of large, mononuclear, c-Kit–positive mast cells in SpA synovitis.

Figure 1. Synovial infiltration with mast cells in patients with spondylarthritis. Immunostaining of the inflamed synovial tissue for CD117 revealed the presence of large, mononuclear, c-Kit–positive cells in the synovial sublining (A). Consecutive stainings of single sections of synovial tissue showed the colocalization of anti-CD117 (B) with toluidine blue staining (C), which indicates the presence of mast cell granules. Double immunofluorescence analysis for anti-CD117 (in green) (D) and mast cell tryptase (in red) (E) confirmed the colocalization (F).
Increased synovial infiltration with mast cells in peripheral SpA

We next aimed to investigate whether synovial infiltration with mast cells was specifically increased in patients with SpA, by performing a systematic comparison of the inflamed synovium from patients with SpA and patients with RA. As SpA is phenotypically heterogeneous, and as it remains uncertain whether the different subtypes display the same cellular and molecular characteristics, we analyzed synovium from patients with nonpsoriatic SpA and patients with PsA separately. The disease groups were well matched for the global level of synovial inflammation, as assessed by the presence of infiltrating CD3+ T cells, CD20+ B cells, and CD68+ macrophages (Figures 2A–C). Moreover, the significant increase in CD163+ macrophages in the synovial sublining of patients with nonpsoriatic SpA ($P = 0.048$) as well as patients with PsA ($P = 0.016$), as compared to that of patients with RA (Figure 2D), confirms that the samples used for this study displayed the prototypical histologic features of SpA and RA synovitis (1,2,24,25). When c-Kit was used as a phenotypic marker, we found that the number of infiltrating mast cells was significantly increased in the nonpsoriatic SpA as well as PsA synovial samples as compared to the RA synovium ($P = 0.015$ and $P = 0.001$, respectively), without differences between the SpA subtypes (Figure 2E). A similar analysis of an independent series of synovial biopsy tissue samples obtained from patients with early, untreated disease confirmed that the number of synovial c-Kit–positive mast cells was significantly increased in SpA synovium compared to RA synovium ($P = 0.004$), irrespective of disease duration, treatment, and SpA subtype (Figure 2F).

Contribution of growth factors and cytokines to synovial mast cell infiltration

The increase in synovial mast cells in SpA synovitis when compared to RA synovitis could be due to recruitment of mast cell progenitors from the circulation, maturation of local mast cell precursors, decreased apoptosis of synovial mast cells, or proliferation of resident mast cells. As the ligand for c-Kit, SCF, is a pivotal mediator of the chemotaxis, differentiation, survival, and proliferation of mast cells [26], we analyzed the concentration of SCF in the synovial fluid. Results of ELISA revealed similar levels of SCF in SpA synovial fluid (median 569 pg/ml, IQR 539–681 pg/ml) and RA synovial fluid (median 522 pg/ml, IQR 425–629 pg/ml). The synovial fluid levels of another important factor for mast cell survival, IL-3 [27], were undetectable in the majority of samples, and were not different between SpA and RA synovial fluid (results not shown).

More recently, the novel IL-1 family member IL-33 was shown to be a potent survival factor and activator of mast cells [28,29]. Whereas IL-33 itself was undetectable in the synovial fluid, the concentration of its soluble decoy receptor, ST2, was significantly decreased in SpA synovial fluid (median 19 pg/ml, IQR 0–36 pg/ml) when compared to RA synovial fluid (median 43 pg/ml, IQR 13–61 pg/ml; $P = 0.043$). Whether the lower level of soluble ST2 potentiates IL-33 signaling in SpA synovitis, and thereby contributes to mast cell infiltration and/or survival, remains to be functionally investigated.
INTERLEUKIN‐17–POSITIVE MAST CELLS CONTRIBUTE TO SYNOVIAL INFLAMMATION IN SPONDYLARTHRITIS

Absence of pronounced mast cell degranulation in SpA synovitis

As one of the major effector functions of mast cells is the rapid release of preformed inflammation mediators by degranulation, we assessed whether the increased numbers of synovial mast cells in SpA synovitis, as compared with RA synovitis, was paralleled by an increase in degranulation. As mentioned above, a majority of the c-Kit–positive mast cells from patients with SpA synovitis coexpressed granules, as shown by double immunostaining of the synovial tissue with toluidine blue and tryptase (Figure 1), suggesting that these cells are not degranulated. Accordingly, not only was the number of c-Kit–positive cells increased, but also the number of toluidine blue–positive cells was significantly increased in SpA synovial tissue as compared to RA synovial tissue, as indicated by the semiquantitative score of cellular infiltration (median 1, IQR 0.5–2 versus median 0.5, IQR 0–1; \( P = 0.030 \)). Moreover, similar levels of tryptase (median 6.2 ng/ml, IQR 3.2–8.7 ng/ml versus median 5.8 ng/ml, IQR 2.9–8.7 ng/ml) and histamine (median 18.0 ng/ml, IQR 12.5–23.3 ng/ml versus median 19.6 ng/ml, IQR 14.0–36.6 ng/ml) were observed in the synovial fluid of patients with SpA and the synovial fluid of patients with RA. Taken together, these results suggest that the increased presence of mast cells is not associated with increased degranulation in SpA synovitis.
Increased IL-17 expression by synovial mast cells in SpA

Besides their specific ability to release proteases and histamine by degranulation, mast cells are increasingly recognized for their production of cytokines, such as TNF and IL-1β [11,17]. More recently, synovial mast cells were demonstrated to express and produce IL-17 in RA synovitis [18]. As IL-17 is emerging as an important pathogenic cytokine in SpA [30], we investigated whether synovial mast cells express IL-17 in SpA. Double immunofluorescence analysis with a polyclonal antibody directed toward IL-17A (but with potentially some cross-reactivity with IL-17F) showed clear colocalization with mast cell tryptase in the SpA synovium (Figure 3A), thus confirming and extending the recent findings in RA synovitis. Quantification of the double stainings, however, showed that synovial mast cells were significantly more frequently expressing IL-17 in SpA (median 66%, IQR 40–84%) than in RA (median 26%, IQR 14–40%; P = 0.015) (Figure 3B). Moreover, mast cells represented a median of 63% (IQR 37–90%) of the IL-17–positive cells in SpA synovitis, as compared to a median of 26% (IQR 10–59%) in RA synovitis (P = 0.036) (Figure 3C).

Additional double stainings to investigate which other cell populations express IL-17A in SpA synovitis revealed a clear colocalization with CD15, a marker for neutrophils (Figure 3D). In contrast to mast cells, however, quantification of the stainings indicated that the relative contribution of neutrophils to the total pool of IL-17–expressing cells was similar between SpA (median 50%, IQR 26–70%) and RA (median 47%, IQR 16–69%) synovial tissue (Figure 3E). There was no colocalization of IL-17 with CD3, a marker for T lymphocytes, in SpA synovitis (Figure 3F). Taken together, these results indicate that mast cells are by far the most abundant IL-17–expressing cell population in SpA synovitis.

Reduction of synovial inflammation by imatinib mesylate

The increased synovial infiltration with mast cells and the augmented expression of IL-17 by mast cells in SpA synovial tissue suggest that these cells may contribute to the inflammatory process. To test this hypothesis, fresh synovial biopsy tissue obtained from the inflamed peripheral joints of patients with SpA (n = 12) was cultured for 3 days ex vivo in the presence or absence of imatinib mesylate. This treatment acts as a potent inhibitor of the tyrosine kinase activity of c-Kit, and has been shown to induce apoptosis of synovial mast cells [31]. Blockade of c-Kit by imatinib mesylate resulted in a significant decrease in the production of IL-17, as measured by ELISA, in the culture supernatants (P = 0.041) (Figure 4A). In contrast, there was no effect on the release of histamine (Figure 4B), thus supporting the histologic findings and observations in the synovial fluid indicating that there is a lack of marked mast cell degranulation in SpA synovitis.

Interestingly, the secretion of other proinflammatory mediators, such as IL-6 (P = 0.027) (Figure 4C) and IL-8 (P = 0.003) (Figure 4D), was also significantly decreased by imatinib mesylate in these ex vivo synovial biopsy tissue cultures. This effect was not only due to diminished secretion, but also due to reduced production, since a similar extent of suppression was seen at the mRNA level, as revealed by qPCR analysis of the treated biopsy tissue as compared to the untreated samples (Figures 4E and F). Taken together, these findings indicate that imatinib mesylate, a potent tyrosine kinase inhibitor affecting c-Kit
signal transduction and inducing mast cell apoptosis, profoundly downregulates synovial inflammation ex vivo.

Absence of modulation of the mast cell/IL-17 axis by effective TNF blockade

Trials with TNF blockers have demonstrated that TNF is an important driver of SpA synovitis [32–34]. As TNF also promotes SCF production by synovial fibroblasts [35], we aimed to assess whether the synovial infiltration with mast cells, as well as the IL-17 expression by mast cells, is a secondary phenomenon that occurs downstream of TNF-driven inflammation in SpA. Analysis of paired synovial biopsy tissue samples obtained before and 12 weeks after clinically effective treatment with etanercept showed no overall decrease in the number of mast cells (Figure 5A), but a clear decrease in the number of IL-17–expressing cells ($P = 0.008$) (Figure 5B).

To clarify whether this decrease could be attributed to reduced expression of IL-17 by the mast cells or to an effect on other cell populations expressing IL-17, we performed and quantified double stainings for mast cell tryptase and IL-17. The percentage of mast cells expressing IL-17 was not affected by treatment with etanercept (Figure 5C), whereas the number of IL-17–positive cells coexpressing mast cell tryptase was significantly increased after TNF blockade ($P = 0.007$) (Figure 5D). Thus, TNF blockade does not affect

Figure 3. Expression and production of interleukin-17 (IL-17) by synovial mast cells. A, Colocalization of IL-17 (in green) and mast cell tryptase (in red) in synovial samples from patients with spondylarthritis (SpA) synovitis. B, Percentage of tryptase-positive mast cells expressing IL-17 in the synovium from patients with SpA compared to the synovium from patients with rheumatoid arthritis (RA). C, Percentage of IL-17–positive cells expressing mast cell tryptase in SpA synovium compared to RA synovium. D, Colocalization of IL-17 (in green) and CD15 (in red) in SpA synovium. E, Percentage of IL-17–positive cells expressing CD15 in SpA synovium compared to RA synovium. F, Absence of colocalization of IL-17 (in green) and CD3 (in red) in SpA synovium. Arrows in A and D indicate colocalization. Bars show the median and interquartile range. * = $P < 0.05$. 
Figure 4. Effect of targeting c-Kit by imatinib mesylate in \textit{ex vivo} cultures of synovial biopsy tissue from patients with spondylarthritis. A–D, Culture supernatants from paired samples of synovial tissue that were treated with imatinib mesylate or left untreated (control) were assessed by enzyme-linked immunosorbent assay for the secretion of interleukin-17 (IL-17) (A), histamine (B), IL-6 (C), and IL-8 (D). E and F, Messenger RNA was extracted from the synovial biopsy tissue and assessed by quantitative polymerase chain reaction for the transcription of IL-6 (E) and IL-8 (F). $^*$ = $P < 0.05$; $^{**}$ = $P < 0.01$.

DISCUSSION
A series of studies in the 1980s and 1990s provided ample evidence of the presence of mast cells in the inflamed synovium of RA patients [for review, see refs. 12–14]. Analysis of tissue specimens obtained during joint replacement surgery indicated a 6–25-fold increase in the number of mast cells in RA patients as compared to patients with osteoarthritis. The present study demonstrated that mast cell infiltration is even more pronounced in SpA.

the synovial infiltration with IL-17–expressing mast cells, but can reduce the infiltration by other IL-17–producing cells, particularly IL-17–positive neutrophils [32–34].
synovitis when compared to RA synovitis, as observed in two independent series of synovial biopsy samples obtained from actively inflamed joints. This difference appears to be specific and not related to a higher global degree of inflammation in SpA, as the numbers of infiltrating macrophages and lymphocytes were similar in both diseases. Moreover, this difference was already observed in patients with early and untreated disease, suggesting that the difference cannot be explained by confounding due to differences in treatment and/or longstanding inflammation. Finally, the marked mast cell infiltration was observed in each of the SpA subtypes, and no difference was observed between psoriatic and nonpsoriatic SpA. The latter point is in agreement with the results from a series of other studies comparing cellular or molecular features of synovial inflammation in different SpA subtypes [4,24,36,37].

The marked, specific, and reproducible increase in mast cells in peripheral SpA raises two additional questions. First, the histologic approach allows evaluation of peripheral SpA, but it remains unknown whether this feature would also extend to axial disease. It also remains to be investigated whether a similar increase in the extraarticular manifestations of SpA, such as psoriasis and gut inflammation, can be observed. These issues are currently under investigation. Second, it is still unclear why the number of mast cells is increased in SpA synovitis. Theoretically, this could be due to increased influx of circulating precursors,
augmented maturation, local proliferation, and/or prolonged survival in the synovial environment. Detailed analysis of these different possibilities would require a more dynamic approach than can be achieved with histologic analysis of the inflamed synovium. In our current preliminary attempt to identify the factors that could contribute to the increased mast cell infiltration, we were not able to obtain any clear clues to indicate that SCF and IL-3 are differentially expressed in SpA and RA synovium, but we did find a small but significant difference in expression of the soluble decoy receptor for IL-33. The exact role of these different factors in SpA synovitis remains to be investigated in more detail.

Mast cells are pleiotropic cells with a wide variety of potential functions, the best known being the rapid release of preformed mediators by degranulation. It has been established that synovial mast cells degranulate to a limited extent in RA synovitis [12–14], as histamine and tryptase are readily detectable in the synovial fluid. The synovial fluid levels of these mediators were not increased in patients with SpA compared to patients with RA, suggesting that, if anything, the degree of degranulation would, rather, be decreased in SpA. Accordingly, double stainings showed that a large majority of the c-Kit–positive mast cells contained granules and coexpressed tryptase, and ex vivo targeting of the synovial mast cells by imatinib mesylate did not affect the release of histamine in the culture supernatant. Even though mediators, such as tryptase and VEGF, contained in the granules may contribute to the marked angiogenesis found in SpA synovium [24,38], these findings, taken together, seem to indicate that degranulation is not a major function of mast cells in SpA synovitis.

Besides degranulation, mast cells are increasingly recognized to play a major role in innate immune defense, as indicated by their production of a variety of cytokines [39,40]. Interestingly, the production and secretion of cytokines are regulated in a manner different from the degranulation function [40–42]. Based on the recent description of IL-17 production by mast cells in inflammatory diseases [19,43,44] and considering the emerging role of IL-17 in SpA [30], we focused on the expression and production of IL-17 by mast cells in SpA synovitis. Our results confirmed not only that synovial mast cells express IL-17, but also that both the absolute number of mast cells and the proportion of synovial mast cells expressing IL-17 is significantly increased in SpA synovitis when compared to RA synovitis. Accordingly, mast cells were the main IL-17–expressing cell population in SpA synovitis. This was confirmed by the fact that targeting mast cells with imatinib mesylate significantly affected the production of IL-17 in synovial biopsy tissue, which thus indicates that mast cells not only express IL-17, but also secrete IL-17.

Although these data are compatible with a functional role for mast cells in synovial inflammation in SpA, a number of issues remain to be investigated. First, as in previous studies [18,43,44], we used a polyclonal goat antibody to IL-17A that can also cross-react, to a certain degree, with IL-17F. Even though the ELISA used for the ex vivo biopsy experiments was specific for IL-17A, and previous in vitro experiments have demonstrated the production of IL-17A by human mast cells [18], it still remains to be formally demonstrated which IL-17 isotype is produced by mast cells in the synovial tissue.

Second, mast cells represent ~70% of the IL-17–expressing cells in SpA synovitis. The findings from our additional double stainings suggested that the other IL-17–expressing
cells are mainly CD15+ neutrophils, and not CD3+ T cells, but further research should characterize these cells in detail.

Third, we did not investigate herein which other cytokines, besides IL-17, are expressed and produced by synovial mast cells in SpA. Mast cells are able to produce a variety of cytokines, including TNF, IL-1β, and IL-6, all cytokines that may be involved in synovial inflammation [11,17,18]. This warrants a systematic analysis of the full functional potential of mast cells in synovitis.

Finally, it remains to be investigated which signals control the production and secretion of these cytokines by synovial mast cells. In vitro experiments demonstrated that human mast cells can produce IL-17A upon stimulation with IgG complexes, C5a, lipopolysaccharide, and TNF [18]. The absence of marked degranulation suggests that cytokine production by synovial mast cells is not primarily mediated by the binding of immune complexes to Fc receptors. Elucidation of the potential role of innate triggering through Toll-like receptors or nucleotide-binding oligomerization domain–like receptors [41,42] and the role of the IL-23/IL-23 receptor axis in global activation and IL-17 production by synovial mast cells will be of particular interest in the context of the current concepts on the pathogenesis of SpA [10,45].

The results obtained in this study also question whether mast cells would be an appropriate target for treatment in SpA. Four lines of evidence support this concept. First, the mast cell infiltration is a primary feature, rather than a consequence of, synovial inflammation, as it is observed in early disease and is not affected by effective TNF blockade. Second, sulfasalazine, the only disease-modifying antirheumatic drug with proven efficacy in peripheral SpA, has been shown to inhibit degranulation and TNF secretion by mast cells [46,47].

Third, our ex vivo experiments with imatinib mesylate, which has been shown to induce apoptosis of rheumatoid synovial mast cells in the same type of experiments [31], resulted in not only a modulation of IL-17 production, but also a down-regulation of other proinflammatory cytokines, such as IL-6 and IL-8. It needs, however, to be emphasized that imatinib mesylate blocks other factors in addition to c-Kit, such as other tyrosine kinases, in particular the macrophage colonystimulating factor receptor c-Fms, which is expressed on macrophage subtypes, and the PDGF receptor, which is expressed on synovial fibroblasts [48,49]. Therefore, it is possible that the effects observed in the ex vivo synovial tissue cultures may not be solely due to modulation of mast cells.

Fourth, an open-label pilot trial with imatinib mesylate in 6 patients with SpA showed a pronounced effect on the signs and symptoms of both peripheral and axial disease [50]. Moreover, there was a 50–60% reduction of the acute-phase response over a 3-month treatment period. Although these clinical data need to be confirmed in larger, placebo-controlled trials and, again, this approach may also affect other cell types besides synovial mast cells, this proof-of-concept trial warrants further clinical investigation of mast cells as a potential therapeutic target in SpA.

In conclusion, our findings indicating the specifically increased infiltration and IL-17 expression of mast cells in SpA synovitis and the profound effect of c-Kit inhibition on synovial inflammation suggest that mast cells contribute to SpA synovitis. Thus, mast cells could be considered to be an attractive therapeutic target in patients with peripheral SpA.
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. Baeten 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.

Study conception and design
Noordenbos, Yeremenko, Gofita, van de Sande, Tak, Cañete, Baeten

Acquisition of data
Noordenbos, Yeremenko, Gofita, van de Sande, Cañete, Baeten

Analysis and interpretation of data
Noordenbos, Yeremenko, Gofita, Tak, Cañete, Baeten
REFERENCES


