Macrophage polarization in spondyloarthritis

Ambăruș, C.A.

Publication date
2012

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Absence of a classically activated macrophage cytokine signature in peripheral spondyloarthritis, including psoriatic arthritis.

Bernard Vandooren, Troy Hoogendoorn, Carmen A. Ambarus, Sarah Kunst, Tineke Cantagrel, Nataliya Yeremenko, Maartje Boumans, Rene Lutter, Paul Tarkowski, Dominique L. Baeten.

1 Division of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands and Ghent University Hospital, Ghent, Belgium

2 Division of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

‡Dr. Vandooren and Mr. Hoogendoorn contributed equally to this work.

Arthritis Rheum. 2009 Apr;60(4):766–75.
Absence of a classically activated macrophage cytokine signature in peripheral spondylarthritis, including psoriatic arthritis

Bernard Vandooren¹,‡, Troy Noordenbos²,‡, Carmen A. Ambarus², Sarah Krausz², Tineke Cantaert², Nataliya Yeremenko², Maartje Boumans², Rene Lutter², Paul P. Tak², Dominique L. Baeten¹

¹ Division of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands and Ghent University Hospital, Ghent, Belgium
² Division of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

‡Dr. Vandooren and Mr. Noordenbos contributed equally to this work.

Arthritis Rheum. 2009 Apr;60(4):966-75.
ABSTRACT

Objective: Peripheral spondylarthritis (SpA) is characterized by macrophages that express CD163, a marker of alternative activation (M2). The purpose of this study was to assess whether this differential infiltration with macrophage subsets was associated with a different local inflammatory milieu in SpA as compared with rheumatoid arthritis (RA).

Methods: The effect of SpA and RA synovial fluid (SF) on macrophage polarization was tested in vitro on normal peripheral blood monocytes. SF levels of classically activated macrophage (M1)–derived and alternatively activated macrophage (M2)–derived mediators were analyzed by enzyme-linked immunosorbent assay and multiparameter Luminex bead assay in 47 patients with non-psoriatic SpA, 55 with RA and 15 with psoriatic arthritis (PsA). Paired synovial biopsy samples were analyzed histologically.

Results: SF from SpA patients promoted preferential expression of the M2 markers CD163 and CD200R in vitro, even if SF levels of the prototypical M2-polarizing factors (interleukin-4 [IL-4], IL-13, and IL-10) were not increased as compared with those in RA SF. Despite a similar degree of overall joint inflammation in SpA and RA, SpA synovitis displayed strongly reduced SF levels of M1-derived, but not M2-derived, mediators, such as tumor necrosis factor α (TNFα), IL-1β, IL-12p70 and interferon-γ–inducible protein 10. SF levels of M1-derived mediators correlated well with peripheral joint inflammation in RA, but neither these mediators nor IL-1α and IL-17 did so in SpA. Of interest, the SF cytokine profile in PsA, a more destructive subtype of SpA, was similar to that in non-psoriatic SpA.

Conclusion: The local inflammatory milieu is clearly different in SpA as compared with RA peripheral arthritis. Synovitis in SpA, including that in PsA, is characterized by a selective decrease in M1-derived proinflammatory mediators, such as TNFα and IL-1β.
Absence of a classically activated macrophage cytokine signature in peripheral spondylarthritis

INTRODUCTION
Prominent swelling and inflammation of peripheral joints is a hallmark of both rheumatoid arthritis (RA) and spondylarthritis (SpA), with a preference for large joints of the lower extremities in SpA. A major source of proinflammatory cytokines in the joint are the synovial macrophages, a prominent synovial cell population that correlates well with the degree of inflammatory disease activity in RA [1]. It has become increasingly clear that macrophages are not a homogenous population but one that can be divided into specific, although overlapping, subsets according to their polarization requirements, phenotype, and function [2,3]. Classically activated macrophages (M1) are the main source of soluble proinflammatory cytokines, such as tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β), whereas alternatively activated macrophages (M2) have been implicated in immune regulation, phagocytosis, and tissue remodeling. Of interest, the total number of macrophages is similar in RA and SpA synovitis, but the subset expressing the M2 surface marker, CD163 [4,5], is clearly increased in the latter [6-8]. Moreover, this specific macrophage subset, but not the total number of macrophages, is correlated with disease activity and reflects response to treatment in SpA [9-11].

These data led to the hypothesis that macrophages expressing the M2 marker CD163 dominate in SpA synovitis, in contrast to the dominance of M1 macrophages in RA. To confirm this hypothesis and to test whether the presence of distinct macrophage subsets in both diseases is associated with different local inflammatory milieus, we studied macrophage-polarizing factors and M1-derived proinflammatory mediators in the inflamed joints of patients with non-psoriatic SpA and psoriatic arthritis (PsA) and compared the findings with those in the joints of patients with RA.

PATIENTS AND METHODS

Patients and samples
The study included 55 patients with RA who fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) criteria [12] and 47 patients with SpA who fulfilled the European Spondylarthropathy Study Group criteria [13], but excluding patients with PsA. Although the SpA patients were primarily selected according to the presence of peripheral disease, 32 of the 47 patients had inflammatory back pain and 15 of the 47 patients clearly had sacroiliitis according to conventional radiographs. A separate cohort of 15 patients with PsA according to the CIALsification of Psoriatic ARthritis criteria [14] was also studied. All patients had active disease with effusion of at least 1 knee joint, which was punctured to obtain synovial fluid (SF). The demographic and clinical characteristics are given in Table 1. None of the patients was treated with a biologic agent. All patients gave
In vitro M2 macrophage polarization

Monocytes from healthy volunteers were isolated from peripheral blood by Ficoll gradient centrifugation and plastic adhesion, and subsequently cultured at a concentration of 0.5 × 10^6/ml in Iscove’s modified Dulbecco’s medium supplemented with 10% fetal calf serum. Purity was >95%, as assessed by CD14 staining. Cells were stimulated with increasing concentrations of pooled SF from either 20 RA samples or 20 SpA samples in the presence or absence of exogenous recombinant IL-10 (50 ng/ml; R&D Systems) as a prototypical M2-polarizing factor. After 4 days of culture, the myeloid cells were recovered and analyzed by flow cytometry using fluorochrome-labeled monoclonal antibodies against CD163 (BD PharMingen, San Jose, CA) and CD200R (Serotec, Oxford, UK). Annexin V staining showed 20–35% apoptotic cells in the higher SF concentrations, but without differences between SpA and RA SF.

All experiments were repeated independently with peripheral blood monocytes from 3 different healthy donors. Similar experiments were also performed with single, rather than pooled, SF samples.

Histologic assessment of the synovial tissue

Synovial tissue samples (n=6-8 per patient) were obtained by needle arthroscopy from the inflamed knee joint of 26 SpA and 33 RA patients, as described previously [15]. Paraffin-embedded tissue sections were stained with hematoxylin and eosin. Global cellular

<table>
<thead>
<tr>
<th></th>
<th>RA (n=55)</th>
<th>SpA (n=47)</th>
<th>PsA (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD years</td>
<td>54 ± 14</td>
<td>37 ± 14</td>
<td>42 ± 13</td>
</tr>
<tr>
<td>Sex, no. male/female</td>
<td>17/38</td>
<td>28/19</td>
<td>11/4</td>
</tr>
<tr>
<td>Disease duration, mean ± SD years</td>
<td>5.2 ± 5.2</td>
<td>4.4 ± 7.6</td>
<td>5.1 ± 6.3</td>
</tr>
<tr>
<td>Swollen joint count, mean ± SD</td>
<td>8.8 ± 5.7</td>
<td>2.8 ± 3.3</td>
<td>2.9 ± 3.8</td>
</tr>
<tr>
<td>CRP, mean ± SD mg/liter</td>
<td>48 ± 56</td>
<td>47 ± 65</td>
<td>26 ± 27</td>
</tr>
<tr>
<td>ESR, mean ± SD mm/hour</td>
<td>46 ± 26</td>
<td>43 ± 29</td>
<td>32 ± 29</td>
</tr>
<tr>
<td>No. taking NSAIDs</td>
<td>31/55</td>
<td>32/47</td>
<td>5/15</td>
</tr>
<tr>
<td>No. taking corticosteroids</td>
<td>14/55</td>
<td>3/47</td>
<td>1/15</td>
</tr>
<tr>
<td>No. taking DMARDs</td>
<td>34/55</td>
<td>16/47</td>
<td>10/15</td>
</tr>
</tbody>
</table>

Table 1. Demographic and clinical characteristics of the RA, non-psoriatic SpA and PsA patients*

*RA = rheumatoid arthritis; SpA = spondylarthritis; PsA = psoriatic arthritis; CRP = C-reactive protein; ESR = erythrocyte sedimentation
infiltration, vascularity, and synovial lining hyperplasia were assessed by semiquantitative scoring (scale of 0-3 for each feature) by 2 independent observers (BV and DB) who were blinded to the diagnosis and clinical data, as extensively described and validated previously [6-11]. The scores assigned by the 2 observers never differed by >1 point. In cases of conflicting scores, the mean of the 2 observers’ scores was used.

**SF analysis**
The levels of the following cytokines were determined by enzyme-linked immunosorbent assay according to the manufacturer’s instructions: IL-1β, IL-1 receptor type II (IL-1RII), IL-6, interferon-γ–inducible protein 10 (IP-10), CCL18, TNFα, and transforming growth factor β1 (TGFβ1) (all from R&D Systems, Abingdon, UK). A multiparameter Luminex bead assay was used to assess SF levels of IL-1α, IL-4, IL-10, IL-12p70, IL-13, IL-17, granulocyte–macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and interferon-γ (IFNγ) as indicated by the manufacturer (Invitrogen, Leek, The Netherlands).

**Statistical analysis**
Data are expressed as the mean ± SEM and were analyzed with the parametric Student’s t-test. Correlations between synovial histologic features and SF cytokine levels were calculated by the nonparametric Spearman’s correlation coefficient, since the semiquantitative histologic score was not normally distributed. P values less than 0.05 were considered statistically significant.

**RESULTS**

**Induction of M2 macrophage markers by SpA SF**
We previously reported a selective increase in macrophages expressing the alternative activation marker CD163 in the inflamed joints of patients with SpA as compared with patients with RA [6-8]. Since this preferential polarization in SpA may be due to local factors rather than intrinsic alterations of macrophage differentiation, we first analyzed the effect of SF from SpA patients compared with SF from RA patients on the polarization of healthy donor peripheral blood monocytes in vitro. Data from a representative experiment are shown in Figure 1.

SpA SF consistently induced a dose-dependent up-regulation of the M2 surface marker CD163 (Figures 1A and B). Conversely, RA SF abrogated the expression of CD163, even in the presence of the M2-polarizing factor IL-10 (Figures 1A and B). These data were confirmed by the expression pattern of CD200R, an inhibitory macrophage receptor that is down-regulated during classic macrophage activation [16,17]. The expression of CD200R was up-
regulated by SpA SF but suppressed by maturation in the presence of RA SF (Figure 1C). The down-regulation of CD200R expression by RA SF could not be rescued by exogenous IL-10 (Figure 1D).

Since these experiments were performed with pooled SF (n = 20 patients per disease group), we performed similar experiments with individual SF samples to exclude the possibility that a few outliers among the individual SF samples biased the results obtained with the pool. Whereas none of the RA SF samples induced CD163 up-regulation, most, but not all, SpA SF samples promoted M2 differentiation (data not shown). Taken together, these data demonstrate that, despite interindividual variability, soluble factors differentially present in SpA SF as compared with RA SF strongly promote the expression of M2 markers.

We therefore next assessed whether this finding could be explained by higher SF levels of the prototypical M2-polarizing factors IL-13, IL-4 and IL-10 [18]. The mean ± SEM SF levels of IL-13 were similar in SpA (9.2 ± 2.9 pg/ml) and RA (6.7 ± 1.9 pg/ml), whereas the mean ± SEM SF levels of IL-4 (2.4 ± 0.9 pg/ml versus 31.6 ± 15.9 pg/ml; P = 0.092) and IL-10 (2.8 ± 1.1 pg/ml versus 11.4 ± 2.8 pg/ml; P = 0.006) were even lower in SpA. Alternative activation of macrophages can also be inhibited by exposure to M1-polarizing factors, such as IFNγ and GM-CSF [18]. However, the mean ± SEM SF levels of GM-CSF were not significantly increased in SpA.
Absence of a classically activated macrophage cytokine signature in peripheral spondylarthritis

different between SpA (9.6 ± 6.0 pg/ml) and RA (13.9 ± 5.9 pg/ml) patients, whereas IFNγ was undetectable in almost all SF samples irrespective of the diagnosis. Therefore, either low paracrine levels of IFNγ or other as-yet-unidentified soluble factors contribute to preferential M2 polarization in SpA synovitis.

**Similar levels of local inflammation in RA versus SpA synovitis**

In order to investigate whether the differences in macrophage phenotype in SpA were related to a distinct inflammatory microenvironment in SpA versus RA peripheral joint inflammation, we first ascertained that the global degree of joint inflammation was comparable in both groups. Parameters of systemic inflammation, such as the C-reactive protein (CRP) level and the erythrocyte sedimentation rate (ESR), were similar in both cohorts (Table 1). More importantly, histologic analysis of synovial biopsy samples obtained from the same joint as the SF samples showed that the level of synovial infiltration with inflammatory leukocytes was similar in both groups (mean ± SEM inflammatory cell infiltration score 1.73 ± 0.19 in SpA versus 1.68 ± 0.17 in RA) (Figure 2A). This was confirmed by the fact that the SF levels
of IL-6, which have been shown to reliably reflect the local degree of joint inflammation [19], were similar in SpA (mean ± SEM 9.7 ± 3.8 ng/ml) and RA (11.7 ± 2.1 ng/ml) patients (Figure 2B). Measurement of a prototypical mediator not directly related to macrophage polarization, G-CSF, also showed similar levels in SF from SpA (mean ± SEM 415.2 ± 46.6 pg/ml) and RA (473.4 ± 73.7 pg/ml) patients (Figure 2C). Taken together, these data indicate that the degree of local inflammation was similar in the two cohorts.

Low levels of M1-derived, proinflammatory cytokines in SpA synovitis

M1 macrophages secrete large amounts of the key proinflammatory cytokines TNFα and IL-1β [20]. Despite the similar degree of global joint inflammation, the SF levels of TNFα (mean ± SEM 5.9 ± 1.3 pg/ml versus 19.9 ± 4.4 pg/ml; P = 0.005) (Figure 2D) and IL-1β (mean ± SEM 3.2 ± 1.0 pg/ml versus 24.1 ± 5.3 pg/ml; P < 0.001) (Figure 2E) were markedly lower in SpA than RA. A similar difference was observed for the prototypical M1 cytokine IL-12p70 in SpA versus RA synovitis (mean ± SEM 5.5 ± 1.7 pg/ml versus 15.2 ± 6.6 pg/ml; P = 0.090) [21]. This was not restricted to cytokines, since the IFNγ-driven chemokine IP-10 produced by M1 [22] was also lower in SpA (mean ± SEM 7.0 ± 1.6 ng/ml versus 14.9 ± 2.2 ng/ml in RA; P = 0.006) (Figure 2F).

<table>
<thead>
<tr>
<th></th>
<th>RA</th>
<th>SpA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SF IL-6</td>
<td>Cellular infiltration</td>
</tr>
<tr>
<td>TNFα</td>
<td>r² = 0.293</td>
<td>r² = 0.409</td>
</tr>
<tr>
<td>p = 0.043</td>
<td>0.523</td>
<td>0.407</td>
</tr>
<tr>
<td></td>
<td>0.006</td>
<td>0.043</td>
</tr>
<tr>
<td>IL-1β</td>
<td>r² = 0.409</td>
<td>r² = 0.407</td>
</tr>
<tr>
<td>p = 0.003</td>
<td>0.420</td>
<td>0.420</td>
</tr>
<tr>
<td></td>
<td>0.033</td>
<td>0.033</td>
</tr>
<tr>
<td>IL-12 p70</td>
<td>r² = 0.342</td>
<td>r² = -0.085</td>
</tr>
<tr>
<td>p = 0.035</td>
<td>0.420</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>0.033</td>
<td>ns</td>
</tr>
<tr>
<td>IP-10</td>
<td>r² = 0.542</td>
<td>r² = -0.001</td>
</tr>
<tr>
<td>p &lt; 0.001</td>
<td>0.413</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>0.050</td>
<td>ns</td>
</tr>
</tbody>
</table>

Table 2. Correlation between SF levels of inflammatory cytokines produced by classically activated macrophages and the degree of local joint inflammation, as reflected by SF IL-6 levels and synovial tissue cellular infiltration, in RA and SpA patients*

*SF = synovial fluid; IL-6 = interleukin-6; RA = rheumatoid arthritis; SpA = spondylarthritis; TNFα = tumor necrosis factor α; NS = not significant; IP-10 = interferon-γ–inducible protein 10.
Absence of a classically activated macrophage cytokine signature in peripheral spondylarthritis

In contrast, there were no significant differences in the levels of mediators produced mainly by M2 macrophages, such as TGFβ1 (mean ± SEM 3,500 ± 1,378 pg/ml versus 2,973 ± 823 pg/ml) (Figure 2G), the soluble decoy IL-1RII (mean ± SEM 9,254 ± 939 pg/ml versus 9,522 ± 1,210 pg/ml) (Figure 2H), or the chemokine CCL18 (mean ± SEM 232.7 ± 27.0 ng/ml versus 237.8 ± 27.9 ng/ml) (Figure 2I), between SpA SF and RA SF. These SF cytokine profiles were not influenced by age, sex, disease duration, or concurrent medication (data not shown). Thus, SpA synovitis was characterized by a selective decrease in proinflammatory mediators mainly produced and secreted by M1 macrophages.

**Correlation of M1-derived cytokines with the degree of local joint inflammation in RA, but not SpA**

The absence of high levels of M1-derived cytokines calls into question the degree to which these mediators are the primary drivers of local synovial inflammation in peripheral SpA. Therefore, we assessed the correlations between SF levels of TNFα, IL-1β, IL-12p70, and IP-10 and the degree of local synovial inflammation, as reflected by SF IL-6 levels and by the degree of infiltration of the synovial membrane by inflammatory leukocytes. As expected, there was a significant correlation between all 4 M1-derived mediators and IL-6 levels in the SF as well as cellular infiltration in the synovial membrane of samples obtained from RA patients (Table 2). In sharp contrast, these correlations were completely lost in samples from patients with SpA (Table 2).

Since these data suggest that the synovitis of SpA may be primarily related to other mediators, we also measured 2 other inflammatory cytokines that were proposed to be involved in SpA based on the findings of large genetic studies. Polymorphisms in the gene encoding IL-1α, which is mostly a cell-associated cytokine but can also be found in the extracellular space, has been reported to show a strong association with SpA [23,24]. Whereas this cytokine was clearly detectable in RA SF, it was almost completely absent from SpA SF (mean ± SEM 0.3 ± 0.2 pg/ml in SpA versus 67.9 ± 34.1 pg/ml in RA; P < 0.001). Another inflammatory cytokine that recently became a focus of new interest in SpA based on the genetic association with the IL-23 receptor is IL-17 [25]. SF levels of IL-17 were significantly lower in SpA patients (mean ± SEM 5.4 ± 3.6 pg/ml) than in RA patients (37.9 ± 16.4 pg/ml; P = 0.047) and did not correlate with either the SF levels of IL-6 or the degree of cellular infiltration of synovial tissue. Thus, these data indicate that the degree of peripheral joint inflammation in SpA is related neither to M1-derived cytokines nor to IL-1α and IL-17.

**Similar findings in PsA and SpA, but not in PsA and RA**

Since there was a highly significant difference in M1-derived SF cytokine levels between
non-psoriatic SpA patients and RA patients, we next investigated whether these mediators would allow us to define specific subgroups within SpA. As indicated above, SF levels of TNFα, IL-1β, IL-12p70, and IP-10 correlated with neither the clinical features, such as age, sex, disease duration, and use of corticosteroids or disease-modifying antirheumatic drugs (data not shown), nor the measures of local disease activity (Table 2). They were also not related to systemic disease activity, as assessed by the number of swollen joints, the CRP level and the ESR, or to SpA subtypes (ankylosing spondylitis [AS] versus undifferentiated SpA, presence or absence of axial disease) (data not shown).

To assess whether PsA is similar to other SpA subtypes in terms of the local inflammatory milieu in the peripheral joint, we also analyzed a separate cohort of SF samples from 15 patients with PsA. Whereas PsA SF again showed levels of IL-6 (13.5 ± 4.3 ng/ml) similar to those in SpA and RA SF, the levels of prototypical M1-derived cytokines TNFα, IL-1β, and IL-12p70, as well as the levels of other differentially expressed cytokines, such as IL-10, IL-1α and IL-17, were consistent with those in SpA SF rather than RA SF (Figures 3A–F). Taken together, these results indicate that the local inflammatory milieu in the inflamed peripheral SpA joint is similar in all clinical subtypes of SpA investigated in this study, including PsA.

Figure 3. Analysis of the local inflammatory milieu in inflamed peripheral joints of patients with psoriatic arthritis (PsA) as compared with that in patients with rheumatoid arthritis (RA) and patients with non-psoriatic spondylarthritis (SpA). Shown are synovial fluid levels of tumor necrosis factor α (TNFα) (A), interleukin-1β (IL-1β) (B), IL-12p70 (C), IL-10 (D), IL-1α (E) and IL-17 (F). Values are the mean and SEM.
Absence of a classically activated macrophage cytokine signature in peripheral spondylarthritis

DISCUSSION

Despite the progress in targeted anticytokine treatments for SpA, the inflammatory cytokine milieu of this disease has not yet been fully characterized [26]. Most studies have focused on T helper cytokines, consistently demonstrating an impaired IFNγ signature in SpA T cells [27-30]. With regard to macrophage-derived mediators, only a limited number of studies have analyzed serum levels of proinflammatory cytokines in SpA, indicating an increase in IL-6 and TNFα, but not IL-1β, in SpA patients as compared with healthy controls [31-33]. Of interest, this profile appeared to be more pronounced in patients with peripheral arthritis than in those with pure axial SpA [26]. Based on the fact that IFNγ is an important determinant of M1 polarization [2,3,20-22] and on the association of synovial CD163+ M2 macrophages, but not T lymphocytes, with disease activity in peripheral SpA [9], the present study examined in more detail whether the presence of distinct macrophage subsets in both diseases is associated with different local inflammatory milieus.

Since our previous histologic findings in SpA synovitis suggested an impaired polarization of myeloid cells toward macrophages that express the alternative activation marker CD163 [6-8], we first aimed to confirm this concept in vitro and to analyze whether this polarization is related to local polarizing factors. The main driver of M1 polarization is IFNγ, a Th1 cytokine that has been reported to be decreased in SpA synovitis, including HLA–B27+ reactive arthritis [27,34]. Consistent with this, macrophages derived from the peripheral blood monocytes of AS patients were reported to have an impaired IFNγ signature as compared with that in healthy controls, which could be restored in vitro by incubation with IFNγ [35]. Macrophages isolated from RA SF were reported to have an opposite profile, with a strong IFNγ signature and a broad resistance to the effects of IL-10, one of the major factors for M2 polarization [36,37].

Extending these observations, we demonstrated in the present study that in contrast to RA SF, SpA SF drives the preferential expression of CD163 and CD200R, markers of M2 versus M1 polarization, in vitro. We could not relate this effect to higher levels of prototypical M2-polarizing factors, such as IL-10, IL-4 or IL-13, in SpA SF, but it is not impossible that this effect is at least partly related to lower levels of the main M1-polarizing factor IFNγ in SpA [35]. Even though we could not detect IFNγ levels in SF samples from either RA or SpA patients, this cytokine has very potent paracrine effects at low concentrations. The effects of exogenous IFNγ on SF-induced macrophage polarization, as well as the intrinsic propensity of SpA monocytes to differentiate into a specific macrophage subset, are currently under further investigation in order to understand mechanistically why peripheral SpA is characterized by CD163+ macrophage infiltration.

Independently of these mechanistic aspects, the main finding of the present study was that
soluble M1-derived mediators, such as TNFα, IL-1β, IL-12p70 and IP-10, were 2–5-fold lower in SpA synovitis than in RA synovitis, despite similar levels of overall local inflammation. These data confirm and extend the observations reported by Cañete et al [27], who found similar levels of IL-6, but lower levels of TNFα and IL-1β, in SF samples from 14 SpA patients as compared with those from 11 RA patients [27].

An important issue here is that these cytokines and chemokines are not produced exclusively by M1, and analysis of SF cannot formally identify the cellular source. However, a number of observations support the notion that the observed differences in SF mediators are related to impaired M1 polarization rather than to alterations of other cellular subsets. First, these data are consistent with the previously reported selective increase in the CD163+ subset in patients with SpA synovitis [6-8], as well as with our in vitro polarization experiments. Second, there was a selective decrease in M1 products rather than a global impairment of macrophage function, since we did not observe differences in M2 products between SpA and RA patients. Finally, previous studies failed to detect clear differences in fibroblast-like synoviocyte products, such as matrix metalloproteinases and tissue inhibitors of metalloproteinases in patients with SpA versus RA synovitis [38]. Taken together, these data indicate a clearly distinct local inflammatory milieu in SpA versus RA synovitis that seems to be at least partly related to preferential expression of the alternative activation markers, such as CD163, in patients with peripheral SpA.

The low levels of M1-derived cytokines in SF samples raise the question about the extent to which these mediators drive and/or perpetuate synovitis. Obviously, the mere concentration of inflammatory cytokines in SF does not necessarily reflect their functional contribution, since mediators such as TNF can be expressed and function as membrane-bound, rather than soluble, cytokines and because the functional outcome results from the balance with the level of anti-inflammatory mediators, such as IL-10. TNFα is clearly important in peripheral SpA despite the relatively low SF levels, since TNF blockade has been shown to dramatically improve synovial inflammation [39-41]. In contrast with RA, however, successful TNF blockade in SpA fails to completely abrogate synovial infiltration by B cells and plasma cells [40,41] or to induce genuine long-lasting remission in SpA because interruption of treatment leads to rapid relapse [42].

Together with the present finding that soluble TNFα and other M1-derived mediators were clearly correlated with local synovial inflammation in RA but not in SpA, these data suggest that M1 macrophages are neither the only nor the major drivers of synovial inflammation in SpA. Many other cell types, including M2 macrophages [43], and other soluble mediators, such as IL-7 and neurotrophins [44,45], have been proposed to play a pathogenic role, but direct functional evidence is still lacking. This also holds true for the new T helper cell subset-
Absence of a classically activated macrophage cytokine signature in peripheral spondylarthritis

secrating IL-17, since we failed to detect increased levels of this cytokine in SF from SpA patients. Of particular interest in this context is the fact that IL-1α was undetectable in SpA SF, leading to the novel hypothesis that the genetic association between SpA and IL-1α [23,24] may thus relate to a decrease, rather than an increase, in this proinflammatory cytokine. A final important issue is how the distinct inflammatory milieu in the inflamed SpA joint translates into differences in phenotype and disease progression as compared with that in the inflamed RA joint. In RA, TNFα and IL-1β are important drivers of bone and cartilage damage [46-50]. Although lower levels of these cytokines in the inflamed SpA joint may contribute to the preservation of tissue integrity, no clear differences in downstream destructive processes have yet been demonstrated between SpA and RA [34,46,51]. Alternatively, rather than protecting from tissue destruction, low levels of M1-derived cytokines in SpA may contribute by allowing tissue repair [50,52]. Although it is beyond the scope of the present study to assess the relationship between the inflammatory milieu and tissue remodeling in SpA, since that would require prospective and longitudinal data in larger cohorts, our analysis of PsA is of particular interest in this context. Whereas tissue destruction is modest in SpA as compared with RA, PsA can display both extensive destructive features and marked signs of repair [53]. If the differing structural phenotype between RA and SpA is mainly due to differences in the local inflammatory milieu, one may expect this inflammatory milieu to also be different in PsA. However, all of the cytokines and chemokines tested and the M1-derived proinflammatory factors in particular were similar in PsA and other SpA subtypes. These data confirm our previous histologic findings that PsA resembles non-psoriatic SpA [54] and indicate that other factors contribute to the regulation of inflammation-driven tissue destruction and repair.

In conclusion, SF cytokine profiling revealed clear differences in the local inflammatory milieu in patients with SpA compared with patients with RA peripheral synovitis, despite similar levels of joint inflammation. In particular, the low levels of M1-derived mediators confirmed a functional impairment of this macrophage subset in SpA. How this may contribute to the pathogenesis and structural outcome of peripheral SpA is currently under investigation.

References


Absence of a classically activated macrophage cytokine signature in peripheral spondylarthritis


