Articular adipose tissue resident macrophages in rheumatoid arthritis patients: potential contribution to local abnormalities

Ewa Kontny\textsuperscript{1} and Monika Prochorec-Sobieszek\textsuperscript{1,2}

Abstract

Objectives. The objectives of this study were to characterize macrophages resident in inflamed articular adipose tissue (AAT) and non-inflamed subcutaneous adipose tissue (ScAT) of RA patients and to evaluate the basal and cytokine-triggered secretory activities of these tissues.

Methods. Tissues were obtained from patients undergoing knee joint replacement surgery. The number of total CD68\textsuperscript{\textast}, CD14\textsuperscript{\textast} and CD163\textsuperscript{\textast} macrophages was evaluated by immunohistochemistry. The concentrations of select factors were measured in supernatants from untreated and cytokine-treated tissue explant cultures using ELISA. IL-1\textbeta and TNF were applied as the stimuli.

Results. Paired samples of AAT and ScAT, obtained from the same patients, contained a similar number of macrophages, displaying an M2-skewed phenotype. Both tissues released equivalent amounts of IL-1\textbeta, TNF, IL-10 and macrophage migration inhibitory factor (MIF). However, AAT secreted more chemokines (CCL2, CCL5), cytokines (IL-6, IL-8, IL-1 receptor antagonist [IL-1Ra]), hepatocyte growth factor (HGF) and MMP-3 than ScAT. Basal secretion of adipocytokines was not patient specific. Except for HGF and MIF, cytokine treatment up-regulated the release of these factors from both tissues, but also upon stimulation AAT produced more IL-6, IL-8 and IL-1Ra than ScAT.

Conclusion. The secretory activity, reflecting cell activation status but not phenotype or the number of macrophages, discriminates rheumatoid AAT from ScAT. By releasing various factors possessing chemotactic, proinflammatory, anti-inflammatory and tissue degrading activities, AAT resident macrophages may drive and control local pathological processes.

Key words: macrophages, articular adipose tissue, adipocytokines, rheumatoid arthritis.

Introduction

Macrophages are innate immune cells that differentiate from blood circulating monocytes recruited into tissues. Under physiological conditions these cells promote tissue homeostasis by producing trophic factors, clearing debris and preventing excessive inflammatory response to environmental stresses. Moreover, macrophages play a crucial role in innate and adaptive immune response, are major mediators of inflammation and are critical drivers in many inflammatory diseases [1]. In response to microenvironmental cues, macrophages undergo functional polarization, resulting in the generation of classical (M1) or alternative (M2) subpopulations [2, 3]. The M1 phenotype is characterized by high production of proinflammatory cytokines, strong microbicidal activity and promotion of T helper 1 immune response, while M2 macrophages are associated with tissue remodelling and have immunoregulatory functions. Many signals (e.g. anti-inflammatory cytokines, including IL-10 and IL-4, glucocorticoid hormones, apoptotic cells and immune complexes) shift differentiation into M2 or M2-like pathways, therefore the M2 subpopulation includes several subsets that share some properties but may differ functionally. In vitro studies indicate that differentiation into M1- or M2-type cells is accompanied by changes in surface molecules...
expression. Some of M2 polarizing factors (e.g. glucocorticoids, IL-10) up-regulate CD163 and therefore this molecule is recognized as an M2 cell marker. Although other cytokines (e.g. IL-4) down-regulate CD14, M2 cells may retain this molecule expression as well [4–7]. In contrast, expression of a pan-macrophage marker, CD68, is similar on M1- and M2-type cells [7]. CD163, a scavenger receptor expressed selectively on monocytes and macrophages, binds and mediates the clearance of haemoglobin–haptoglobin (Hb–Hp) complexes formed at sites of local tissue destruction [8]. Although binding of Hb–Hp complexes usually triggers production of anti-inflammatory factors, the induction of proinflammatory cytokines has also been reported [8, 9]. Hence CD163 is recognized as an immunoregulatory molecule. During the late resolution phase of inflammation, CD163+ cells constitute the predominant macrophage population implicated in wound-healing, angiogenesis and protection of the host from an overwhelming inflammatory response [8]. Another marker of monocyte/macrophage lineage, the CD14 molecule, plays a critical role in immune defence because it forms part of the receptor for bacterial lipopoly-saccharide. Importantly, M1 and M2 macrophages have the capacity to switch from one phenotype to another and in vivo coexistence of cells with unique or mixed phenotypes is observed [2, 3, 4, 10, 11].

In the RA joint, synovial macrophages participate in the hyperplasia and chronic inflammation of the SM and in the destruction of adjacent cartilage and bone [12]. These cells reside in the intimal lining and sublining layers of the SM. Subsynovial macrophages display a mixed M1/M2 phenotype, play proinflammatory and prodestruc-tive roles and are considered as the most reliable biomarker for disease severity and response to therapy [12]. On the other hand, intimal lining macrophages have an M2-like phenotype (CD163+/CD14+/−), and presumably represent mature resident macrophages. However, their role in joint pathology is unknown [13].

Recently we characterized a secretory profile of rheumatoid articular adipose tissue (AAT) and reported that AAT-originated factors intensified pathogenic activities of fibroblast-like synoviocytes [14]. In adipose tissue (AT), cytokines and growth factors (collectively known as adipocytokines) are produced principally by AT macrophages (ATMs) [15]. Obesity increases the number of ATMs and induces a switch from an M2-like phenotype, characteristic for normal AT, to an M1 state, which is reflected by elevated secretion of proinflammatory chemo-kines and cytokines [16, 17]. However, there are also reports showing that human ATMs have M2-like surface markers but produce excessive amounts of proinflamma-tory and anti-inflammatory factors [18–20].

By analogy, we assumed that rheumatoid AAT contains ATMs as well. Because data concerning the phenotype and role of these cells are missing, the aim of this study was to characterize AAT macrophages by quantitative estimation of CD68+, CD14+ and CD163+ cells, as well as by a more detailed description of the basal and induced secretory activity of AAT. As a control we used non-inflamed subcutaneous periarticular adipose tissue (ScAT) obtained from the same RA patients.

Patients and methods

Patients

A group of 47 patients (41 female, 6 male) who fulfilled the revised criteria for the diagnosis of RA stages III–IV was included in this study [21]. The mean (range) patient age, disease duration and disease activity score (DAS28) was 55.8 (29–68) years, 17.3 (1–34) years and 4.32 (2.89–6.23), respectively. Patients were treated with MTX and steroids; none had received biological therapy. MTX treatment was discontinued 1 week before surgery.

Tissue specimens (~1 g) were obtained from the same RA patients at the time of total knee joint replacement surgery, performed as a normal part of clinical care. Tissue specimens were taken from the Hoffa’s infrapatel-lar fat pad (AAT) and from the site of skin closure with sutures (ScAT). All patients gave their written informed consent according to the Declaration of Helsinki and the study was approved by the Ethics Committee of the Institute of Rheumatology, Warsaw, Poland.

Immunohistochemistry of rheumatoid AAT and ScAT

Freshly obtained AT samples were fixed in 10% formalin, routinely processed and embedded in paraffin wax. Immunohistochemical staining with monoclonal antibodies against CD68 (clone PG-M1, dilution 1:50; Dako Denmark A/S, Glostrup, Denmark), CD163 (clone 10D6, dilution 10D6, dilution 1:100; Novocastra, now part of Leica Microsystems, Wetzlar, Germany) and CD14 (clone 7, dilution 1:50; Novocastra) was applied to 5 μm thick paraffin-embedded sections to identify and quantify macrophages. Staining was performed according to the manufacturer’s instructions and was preceded by a tissue endogenous peroxid-ase activity blocking step. The EnVision Detection System (Dako Denmark A/S) was used for detection. Staining of human tonsil macrophages with antibodies specific to CD68, CD163 or CD14 was done as the positive controls. Negative (isotype) controls were performed using Ready-to-Use FLEX Negative Control Mouse (cocktail of mouse IgG1, IgG2a, IgG2b, IgG3 and IgM; code nr IR750; Dako Denmark A/S). The number of CD68+, CD163+ and CD14+ macrophages was estimated at magnification ×100. All cases were photographed at ×100 magnification with the Olympus BX63 DP72 camera (Olympus) and analysed with the acquisition software of the CellSens program (Olympus). The CD68+, CD163+ and CD14+ cells with macrophage morphology were counted in an area equal to 1.897 mm².

Tissue culture and treatment

Tissue processing, explant preparation and cultures were performed as described previously [14]. Briefly, tissue explants (100 mg/ml/well) were pre-cultured for quiescence (26 h, 37 °C, 5% CO2) in Dulbecco’s modified eagle medium supplemented with antibiotics (50 μg/ml of gentamicin, 100 mg/ml kanamycin). Before stimulation, the
culture medium was replaced with fresh medium and the tissue explants were stimulated for 18 h with recombinant human IL-1β (1 ng/ml) or TNF (10 ng/ml) (both from R&D Systems, Minneapolis, MN, USA). To evaluate spontaneous (untreated cultures) and cytokine-triggered secretory activities of tissues, the concentrations of select factors were measured in culture supernatants by ELISA.

ELISAs

The ELISAs for IL-6 and IL-8 were performed as previously described [22]. The evaluations of other factors were done using commercially available ELISA sets: the DuoSets from R&D Systems for monocyte chemoattractant protein (MCP-1/CCL2), regulated on activation, normal T cell expressed and secreted (RANTES/CCL5), hepatocyte growth factor (HGF), IL-1 receptor antagonist (IL-1Ra), macrophage migration inhibitory factor (MIF) and MMP-3 and the Ready-Set-Go sets from eBioscience (San Diego, CA, USA) for IL-1β, IL-10 and TNF. The above assays were performed according to the manufacturer’s protocols.

Statistical analysis

Data were analysed using STATISTICA 6.0 software (StatSoft Inc., Tulsa, OK, USA). The Wilcoxon signed-rank test was applied to compare the results obtained from paired AAT and ScAT samples and to evaluate the effects of stimuli. Correlation was assessed using a Spearman test (r value is shown). P values <0.05 were considered significant. Values are shown throughout the article as the mean (S.E.M.) and/or the median (IQR).

Results

Similarity of resident macrophage population in rheumatoid AAT and ScAT

AAT was massively infiltrated with macrophages that were located in interstitial spaces between adipocytes. The population of ATMs was heterogeneous, composed mostly of CD163+ cells and less numerous CD14+ cells (Figs. 1 and 2; Table 1). A similar picture was observed in ScAT (not shown). The strong positive correlation between CD68+ and CD163+, but weaker between CD14+ and CD68+, as well as between CD14+ and CD163+ cell numbers was found in every tissue (Fig. 2). These results show that in both tested tissues almost all ATMs express CD163, while the expression of CD14 is more limited. A more detailed analysis evidenced no difference between AAT and ScAT with respect to the number and phenotype of ATMs (Table 1). Both tissues contained several times

![Fig. 1 Representative photomicrographs showing AAT resident macrophages.](https://academic.oup.com/rheumatology/article-abstract/52/12/2158/1801911)
more CD163+ than CD14+ ATMs and calculated CD163+/CD14+ cell ratios were equal. In the majority of patients the number of total CD68+, CD163+ and CD14+ macrophages was similar in both tissues and we found positive correlation between them (Fig. 2). Thus macrophages residing in both inflamed AAT (AAT-ATMs) and non-inflamed ScAT (ScAT-ATMs) represent an M2-skewed population of cells.

Rheumatoid AAT is a richer source of adipocytokines than ScAT

The number of total CD68+, CD163+ and CD14+ ATMs was evaluated by immunohistochemistry. The paired AAT and ScAT samples (n = 32) from individual RA patients are identified by a line between points (upper panel). The correlation between the number of ATMs present in AAT and ScAT (upper panel), AAT (middle panel, n = 35) and ScAT (lower panel, n = 32) was assessed using a Spearman test (r value is shown). **P < 0.001; ***P < 0.001; ****P < 0.0001. The differences between the number of ATMs present in AAT and ScAT were not significant (n.s.).

The evaluation of basal secretory activities of AAT and ScAT revealed that both tissues produced similar IL-1β, TNF, IL-10 or moderate (MIF) amounts of some cytokines (Table 1; Fig. 3), while other factors (IL-6, IL-8, IL-1Ra, HGF, MMP-3, MCP1/CCL2, RANTES/CCL5) were produced in greater quantities by AAT than ScAT (Table 1; Fig. 4). Only a part of the AAT and ScAT specimens produced spontaneously detectable amounts of TNF, IL-1β or IL-10 (Table 1) and there was no significant correlation between these cytokine secretions from tested tissues (Fig. 3). Among other adipocytokines, some (MIF, HGF, CCL2, MMP-3) were produced by all AAT and ScAT specimens, while others (IL-6, IL-8, IL-1Ra, CCL5) were released more frequently by AAT than ScAT specimens (Table 1). Only the secretion of MIF, HGF and IL-6 from AAT and ScAT showed a positive (high, moderate and weak, respectively) correlation (Fig. 3). In the majority of patients, basal secretion of chemokines, cytokines, HGF and MMP-3 was greater from AAT than ScAT, no matter whether their releases from tested tissues were correlated or unrelated (Fig. 4). Thus, despite the similarity in ATM
chemokines (MCP-1/CCL2, RANTES/CCL5) was measured by ELISA. Values are the median (IQR) or the mean (S.E.M.).

IL-6, IL-8) and anti-inflammatory cytokines (IL-10, IL-1Ra), growth factor (HGF), tissue degrading enzyme (MMP-3) and contents, basal factor secretion was not patient specific and AAT was a richer source of the majority of adipocytokines as compared with ScAT. Moreover, we failed to find any relationship between the number of resident ATMs and the basal secretory activity of tested tissues, except the inverse correlation of CD163+ cell number in AAT and the release of MMP-3 from this tissue (Fig. 4).

Reactivity of rheumatoid AAT and ScAT to proinflammatory cytokines

To check whether the secretory activity of AAT is a unique feature of this tissue or results from its exposure to an inflammatory microenvironment, we mimicked in vivo conditions by evaluating the reactivity of AAT and ScAT to proinflammatory cytokines, i.e. TNF and IL-1β, known to be overproduced in the rheumatoid joint [23]. In both tissues the secretion of IL-1β was unchanged upon TNF treatment, while TNF release was significantly elevated in the presence of IL-1β. IL-1β and TNF up-regulated the secretion of IL-6, IL-8, IL-10, IL-1Ra and MMP-3 from both tissues and IL-1β exerted a stronger effect than TNF. Moreover, upon IL-1β and/or TNF treatment, AAT produced more IL-6, IL-8 and IL-1Ra than ScAT. In contrast, there was no difference between AAT and ScAT in the cytokine-triggered MMP-3 and IL-10 production. The release of MIF was slightly up-regulated only in cytokine-treated ScAT, while HGF secretion did not change significantly as compared with untreated cultures, except a slight decrease in IL-1β-treated AAT cultures (Fig. 5).

Thus, although both ATs were reactive to proinflammatory cytokines upon stimulation, AAT produced more IL-6, IL-8 and IL-1Ra than ScAT.

Discussion

In this study we found that the AAT-ATM population contains several times more CD163+ than CD14+ cells and thus represents an M2-like subset. In regard to phenotype and number, these cells resemble ATMs present in non-inflamed ScAT (Table 1; Fig. 2). This observation is consistent with other data showing an M2-skewed macrophage population in human ScAT and no phenotypic differences in ATMs from different AT locations [11, 18, 19]. The preferential location of M1 cells around dead adipocytes and M2 cells in fibrotic areas of human ScAT was also reported, suggesting different roles for these cell subsets. Interestingly, obesity was accompanied by further accumulation of M2 cells, which generated an inflamed microenvironment and more fibrosis as a result of exposure to secretory products from adipocytes [20]. AT-derived mesenchymal cells were also reported to support the polarization of human macrophages into M2 type [24]. Hence M2 polarization of human ATMs seems to be determined not only by cytokines, but also by interaction with adipocytes and stromal cells.

Human AT explants and isolated ATMs produce numerous proinflammatory and anti-inflammatory factors [14, 18, 19, 25]. In the present study we confirmed and extended our previous characterization of rheumatoid

### Table 1 Characterization of rheumatoid AAT and ScAT

<table>
<thead>
<tr>
<th></th>
<th>AAT</th>
<th>ScAT</th>
<th>Positive samples</th>
<th>P-value</th>
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</thead>
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<tr>
<td></td>
<td>Median (IQR)</td>
<td>Mean (S.E.M.)</td>
<td>Median (IQR)</td>
<td>Mean (S.E.M.)</td>
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<td>Number of macrophages (n = 32)</td>
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<tr>
<td>CD68+</td>
<td>84.5 (83)</td>
<td>96.3 (12.8)</td>
<td>94.5 (90.5)</td>
<td>112 (12.9)</td>
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<td>CD163+</td>
<td>68.5 (59.5)</td>
<td>81.8 (11.6)</td>
<td>76 (74.5)</td>
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<td>CD14+</td>
<td>16 (15.5)</td>
<td>20.9 (3.6)</td>
<td>20.5 (19.5)</td>
<td>24.8 (4.4)</td>
</tr>
<tr>
<td>CD163+/CD14+</td>
<td>4.3 (3.9)</td>
<td>6.9 (1.7)</td>
<td>4.5 (5.5)</td>
<td>7.7 (2.3)</td>
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<td>Basal secretion, pg/100 mg tissue</td>
<td></td>
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<td>TNF (n = 47)</td>
<td>0 (0.2)</td>
<td>4.9 (2.8)</td>
<td>0 (0)</td>
<td>0.9 (0.4)</td>
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<td>IL-1ß (n = 47)</td>
<td>0 (2)</td>
<td>4.7 (2.6)</td>
<td>0 (1.7)</td>
<td>1.1 (0.2)</td>
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<tr>
<td>IL-6 (n = 45)</td>
<td>325 (1490)</td>
<td>2780 (978)</td>
<td>45 (277)</td>
<td>790 (302)</td>
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<tr>
<td>IL-8 (n = 45)</td>
<td>51 (1648)</td>
<td>3320 (1528)</td>
<td>0 (131)</td>
<td>370 (157)</td>
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<tr>
<td>MIF (n = 45)</td>
<td>157 (306)</td>
<td>484 (145)</td>
<td>218 (392)</td>
<td>480 (111)</td>
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<tr>
<td>IL-10 (n = 46)</td>
<td>0.22 (2.4)</td>
<td>2.1 (0.68)</td>
<td>0 (3.5)</td>
<td>2.3 (0.6)</td>
</tr>
<tr>
<td>IL-1Ra (n = 46)</td>
<td>205 (439)</td>
<td>492 (131)</td>
<td>127 (215)</td>
<td>208 (36)</td>
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<tr>
<td>HGF (n = 24)</td>
<td>2980 (4159)</td>
<td>4871 (934)</td>
<td>1803 (1494)</td>
<td>2645 (461)</td>
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<td>MMP-3 (n = 25)</td>
<td>4202 (6153)</td>
<td>5997 (1066)</td>
<td>1330 (2030)</td>
<td>2381 (539)</td>
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<td>MCP1/CCL2 (n = 32)</td>
<td>1146 (914)</td>
<td>1358 (145)</td>
<td>618 (719)</td>
<td>796 (156)</td>
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<td>RANTES/CCL5 (n = 29)</td>
<td>2.8 (7.7)</td>
<td>30.3 (20.4)</td>
<td>0 (1.7)</td>
<td>2.2 (0.8)</td>
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</table>

The number of macrophages was evaluated by immunohistochemistry, Basal secretion of indicated pro- (TNF, MIF, IL-1ß, IL-6, IL-8) and anti-inflammatory cytokines (IL-10, IL-1Ra), growth factor (HGF), tissue degrading enzyme (MMP-3) and chemokines (MCP-1/CCL2, RANTES/CCL5) was measured by ELISA. Values are the median (IQR) or the mean (S.E.M.). Tissue specimens were obtained from the same RA patients; n, number of patients. Comparisons between AAT and ScAT were analysed by the Wilcoxon signed-rank test (P-value); *statistically significant.
AAT secretory activity [14]. In particular we found that higher basal release of several factors (CCL2, CCL5, IL-6, IL-8, IL-1Ra, HGF and MMP-3) discriminated rheumatoid AAT from ScAT (Table 1). Moreover, we noticed that spontaneous adipocytokine secretion was not patient specific and in general was not related to the number of resident ATMs (Figs. 3 and 4). Thus we supposed that higher basal secretory activity of AAT might result from exposure of this tissue to the inflammatory milieu in vivo. We confirmed this assumption by showing that upon in vitro stimulation with proinflammatory cytokines (IL-1β, TNF) AAT retained its greater capability to release IL-6, IL-8 and IL-1Ra as compared with ScAT. Nevertheless, both cytokine-treated tissues responded similarly by up-regulation of IL-10 and MMP-3. In general, IL-1β was more potent in increasing release of these factors, while neither IL-1β nor TNF elevated the secretion of MIF and HGF, at least in AAT cultures (Fig. 5). Based on our previous [14] and present results, we propose that in rheumatoid joints IL-1β produced primarily by synovial cells, triggers TNF secretion from AAT and both these cytokines exert a potent stimulatory effect on AAT.

Certainly IL-1β and TNF should be considered as the general AT activators, as their effects on ScAT secretory activity were also prominent (Fig. 5).

Numerous chemokines are pivotal for ATM recruitment, but CCL2 and CCL5 seem to play a critical role in this process, as both are expressed and secreted by AT from different locations, originate mostly from ATMs and trigger transmigration of blood monocytes through endothelial cells [26, 27]. ATMs are thought to be attracted by both CCL-2 and CCL-5, while T lymphocyte recruitment is dependent on CCL-5 [27]. However, these observations refer to obesity-associated ATM infiltration. The mechanisms underlying ATM accumulation in rheumatoid AAT have not been defined so far. Our results point to CCL2 as being primarily involved in this process, because AAT produced huge amounts of this chemokine and notably less CCL5. In addition, the release of chemokines, especially CCL2, was significantly higher from AAT than ScAT (Table 1). Consistently, more ATMs should have been present in AAT than ScAT. Contrary to expectations, both ATs contained a similar number of these cells (Table 1). In the knee joint, AAT is situated intracapsularly and

**Fig. 3** Adipocytokines produced in similar quantities by AAT and ScAT.

**A** IL-1β 
(\( r = 0.1 \))

**B** TNF 
(\( r = 0.05 \))

**C** IL-10 
(\( r = 0.19 \))

**D** MIF 
(\( r = 0.69^{***} \))
FIG. 4 Adipocytokines produced in greater amounts by AAT than ScAT.

(A–G) The basal secretion of indicated adipocytokines from AAT and ScAT specimens was evaluated by ELISA. The paired AAT and ScAT samples from individual patients are identified by a line between points. The number of paired samples is the same as in Table 1. The data were analysed by the Wilcoxon signed-rank test. The correlation was assessed using a Spearman test (r value); statistically significant values are marked in bold. (H) The inverse correlation between basal MMP-3 release from AAT and the number of CD163⁺ ATMs present in this tissue. *P < 0.05; **P < 0.01; ***P < 0.001.
extrasynovially, but in close contact with SM. Therefore it is likely that AAT-originated CCL2 may attract macrophages mostly to nearby SM and by this way may support local inflammation. However, the above supposition requires further investigation to be confirmed. Although AAT and ScAT released comparable amounts of MIF (Table 1; Fig. 3), this cytokine contribution to ATM recruitment and local inflammation could not be neglected. MIF is a proinflammatory cytokine produced by innate immune cells and adipocytes, has a chemokine-like function and promotes leukocyte migration into inflammatory sites. In RA patients increased systemic and local MIF levels were detected [28]. The present results point to AAT as an additional pool of MIF in the rheumatic joint.

The involvement of IL-6 in RA pathogenesis is well proved and IL-6 inhibition is beneficial to patient, while the role of IL-8 consists of promotion of angiogenesis and attracting and activating neutrophils [29, 30]. Consistent with our previous findings [14], we noted that rheumatoid AAT produced greater amounts of these cytokines than ScAT. In contrast, both AAT and ScAT secreted minute amounts of anti-inflammatory IL-10, even upon exposure to proinflammatory cytokines (Table 1; Fig. 3). These results question the importance of IL-10 in M2 polarization in these tissues. Importantly, AAT secreted considerable and greater amounts of IL-1Ra and HGF than ScAT (Table 1; Fig. 4). Because these factors possess anti-inflammatory activities, we suggest that rheumatoid AAT may also control local inflammation. IL-1Ra is a natural antagonist of IL-1, and is used as a biologic drug in human medicine, including RA patients. HGF originates from stromal cells, is important for inducing self-repair response in numerous organs, in vitro inhibits proinflammatory cytokine production in macrophages and suppresses collagen-induced arthritis in mice [31]. Nevertheless, the role of HGF in RA is unclear, as baseline plasma HGF levels have been reported to predict joint damage in RA patients, suggesting its prodestructive role [32].

MMPs, the extracellular matrix-degrading enzymes, are implicated in both physiological (wound healing and tissue repair) and pathological processes. High levels of several metalloproteinases have been found in rheumatic joints and MMP-3 was particularly up-regulated [33]. Recent data denote serum MMP-3 as a strong predictor of radiographic progression in RA patients and thus emphasize the role of this enzyme in joint destruction [34]. In this study we observed a greater basal release of MMP-3 from AAT than ScAT and a further increase of MMP-3 secretion from both
tissues upon cytokine exposure (Table 1; Fig. 5). Thus it is likely that AAT contributes to joint tissue destruction. However, the inverse correlation between CD163+ AAT-ATM number and basal MMP-3 secretion from AAT questions such a notion (Fig. 4). This striking finding suggests an ability of CD163+ AAT-ATMs to attenuate MMP-3 synthesis, e.g. by IL-1Ra delivery. Another possibility is that the CD163+ macrophage population contains a particular subset that expands with accumulation of ATMs and is endowed with such inhibitory activity. Certainly further studies are required to clear up this relationship. Nevertheless, our results somewhat support the latter supposition, because we found that the CD14+ AAT-ATM number inversely correlated with disease duration ($r = -0.57; P < 0.001; n = 34$; data not shown), indicating alterations in the composition of AAT-ATMs. Altogether our results allow us to conclude that in the rheumatic joint AAT exerts not only proinflammatory, but also anti-inflammatory effects. Because we failed to find a correlation between AAT-ATM number and the release of other factors, except MMP-3, we suggest that the activation status is more important in determining AAT secretory activity.

The limitation of our study is that we did not analyse isolated ATMs. However, because only small amounts of tissue specimens could be obtained from the patients, such analysis was impossible to perform. In addition, mesenchymal stem cells isolated from the AAT of RA patients spontaneously produce small amounts of IL-6, IL-8 and MMP-3, and did not secret either HGF or IL-10 (our own unpublished observations). Therefore we consider ATMs to be the main cell population responsible for the secretory activity of rheumatoid AAT.

In conclusion, we propose that in the rheumatoid joint not only synovial, but also AAT resident macrophages participate in pathological processes. For the first time we have identified these cells as an M2-skewed population. We have found that the basal release of several factors (CCL2, IL-6, IL-8, IL-1Ra, HGF and MMP-3), reflecting resident macrophage activation, but not their phenotype nor number, discriminates inflamed AAT from non-inflamed ScAT. Considering the biological activity of these factors, it is likely that they may drive and regulate local pathological processes.

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