Peroxisome proliferator-activated receptor-gamma expression in monocytes/macrophages from rheumatoid arthritis patients: relation to disease activity and therapy efficacy—a pilot study

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Abstract

Objectives. Peroxisome proliferator-activated receptor-gamma (PPARγ) is expressed by different cell types in the joints and plays a relevant anti-inflammatory role in various diseases. This pilot study aimed to evaluate PPARγ expression in monocytes/macrophages isolated from RA patients as compared with healthy subjects, the relationships between PPARγ expression, MMP-9 activity and disease, and the influence of therapy with anti-rheumatic drugs on these parameters.

Methods. Thirty RA patients of both sexes (treated with CSs and MTX, mainly) and 15 healthy volunteers were enrolled in this study. Disease severity was evaluated by the 28-joint disease activity score (DAS-28). Monocytes and monocyte-derived macrophages (MDMs) were isolated by standard procedures. PPARγ protein and mRNA expression were assessed by immunoblotting and real-time PCR, respectively; MMP-9 activity was determined by gelatin zymography. Moreover, we checked the ability of 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ, a PPARγ agonist), MTX and methylprednisolone (MP) to affect PPARγ expression and lipopolysaccharide (LPS)-induced MMP-9 activity.

Results. Monocytes/MDMs from RA patients have significantly enhanced PPARγ expression (both protein and mRNA) and MMP-9 activity as compared with healthy donors. Interestingly, cells from patients with less active disease (DAS-28 <3.2) present higher PPARγ protein expression and lower MMP-9 activity than RA patients with DAS-28 >3.2. At therapeutic concentrations, MTX and MP increase in vitro PPARγ protein expression and inhibit LPS-induced MMP-9 activity.

Conclusion. PPARγ expression in human monocytes/MDMs could represent an indicator of disease activity and therapy efficacy in RA because patients with a DAS-28 score <3.2 show the highest expression.

Key words: rheumatoid arthritis, DAS-28, monocyte/macrophage, PPAR-gamma, methotrexate, methylprednisolone, matrix metalloproteinase-9.

Introduction

RA, a systemic inflammatory and autoimmune disease of unknown aetiology, is characterized by chronic inflammation of the synovium and erosions preferentially involving peripheral joints. Monocytes/macrophages infiltrate into the joints and produce various bioactive factors, including cytokines and MMPs, that cause persistent inflammation and lead to joint destruction [1]. Several studies have highlighted the anti-inflammatory properties of peroxisome proliferator-activated receptor-gamma (PPARγ)
agonists, either the endogenous 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ) or the synthetic thiazolidinediones (TZD), in experimental models of arthritis [2–5] and in various inflammatory cells [6–10]. Previous reports have also documented that PPARγ is expressed at both mRNA and protein levels by major cell populations in joints [6–9, 11–13]. Moreover, we found a constitutively enhanced PPARγ expression in both monocytes and macrophages from patients with coronary artery disease as compared with healthy subjects [12, 14]. At the cellular level, 15d-PGJ and TZD inhibit the transcriptional induction of genes that contribute to joint pathology, e.g. TNF-α [6–8], IL-1 [6], gelatinase B [7], inducible nitric oxide synthase and MMP-13 [9]. Interestingly, up-regulation of innate immune pathways, including IL-6, toll-like receptor/IL-1 receptor and PPAR signalling, was observed in peripheral blood mononuclear cells from patients with systemic JIA [15]. Therefore this pilot study aimed to evaluate whether (or not) PPARγ expression was up-regulated in monocytes/macrophages from RA patients in comparison with healthy volunteers and its possible relationship with MMP-9 activity and disease score activity (based on the 28-joint Disease Activity Score, DAS-28).

**Materials and methods**

**Patients**

Thirty adult RA outpatients (20 females and 10 males) attending the local immuno-rheumatology clinic between January 2010 and January 2011 and 15 healthy donors (10 females and 5 males) were enrolled in this pilot study and gave their informed written consent. The study was approved by the Ethics Committee of Azienda Ospedaliera Maggiore della Carità, Novara. The inclusion criteria for the RA group were as follows: age 25–90 years, no smoking habit, established RA (disease duration 3 years or longer), current RA therapy with MTX, other DMARDs and/or low-dose oral CSs and no other chronic diseases (including hypertension, diabetes and hypercholesterolaemia). RA was diagnosed according to the 1987 criteria of the ACR [16]. For each patient, a complete medical history was obtained and a full physical examination, including joint assessment, was performed. Clinical assessment routinely included the DAS-28 [17], ESR and CRP level, RF and ACPAs. To be recruited in this study, healthy donors had to be non-smokers, drug-free (including paracetamol or aspirin) at the time of the study, age-matched to RA patients and not presenting inflammatory/immune or other chronic diseases. Smokers (both RA patients and healthy volunteers) were excluded from this study because tobacco smoke deeply affects PPARγ expression in monocytes/macrophages [8, 14], as well as the patient’s response to MTX treatment [18]. Blood samples were obtained from each participant at fasting at 9.00 a.m.

**Isolation of monocytes and macrophages from RA patients and healthy donors**

Human monocytes were isolated from heparinized venous blood by standard techniques of dextran sedimentation and Histopaque (density = 1077 g/cm³) gradient centrifugation (400g, 30 min, room temperature) and recovered by thin suction at the interface, as described [8, 12, 14]. Cells were re-suspended in RPMI 1640 medium, supplemented with 5% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine and antibiotics; purified monocyte populations were obtained by adhesion (90 min, 37°C, 5% CO2), non-adherent cells (mainly lymphocytes) being removed by gentle washing. Cell viability (trypan blue dye exclusion) was usually >98%; expression of surface markers was analysed by flow cytometry, yielding >90% pure monocyte populations [8]. Monocyte-derived macrophages (MDMs) were prepared from monocytes by culture (8–10 days) in a 5% CO2 incubator at 37°C in RPMI 1640 medium containing 20% FBS, glutamine and antibiotics, and defined as macrophage-like cells by evaluating surface markers, as described [8, 12, 14]. Our monocyte preparations were 96% CD14+, 62% CD68+ and 99% major histocompatibility complex (MHC) class II (MHCII), whereas MDM preparations were 68% CD14+, 97% CD68+, 78% MHCII++; the lack of CD1a expression demonstrated that there was no differentiation towards dendritic cells.

**PPARγ protein expression and semi-quantitative analysis**

The constitutive expression of PPARγ protein was evaluated in monocytes and MDMs by western blot, as described [8, 12, 14]. Cells were scraped off in radiomunoprecipitation buffer and lysed by sonication; the determination of protein concentration was done with a bicinchoninic acid assay. Protein samples (20 μg) were analysed by SDS-PAGE (10% acrylamide) and electro-blotted on nitrocellulose membrane (Protran, Perkin Elmer Life Sciences, USA). Immunoblots were performed using polyclonal rabbit anti-human PPARγ (Abcam, UK), and monoclonal mouse anti-human β-actin (Sigma, Italy) antibodies; anti-mouse and anti-rabbit secondary antibodies were coupled to horseradish peroxidase. Chemiluminescence signals were analysed under non-saturating conditions with an image densitometer (Versadoc, Bio-Rad, USA). Semi-quantitative evaluation of PPARγ protein was performed by calculating the ratio between its total expression and the expression of the reference housekeeping protein, β-actin [8, 12, 14]. We also evaluated the ability of the endogenous PPARγ agonist 15d-PGJ and of two anti-rheumatic drugs largely used by our RA patients, MTX and methylprednisolone (MP), to affect PPARγ protein expression in vitro. In these experiments, cells from healthy donors were treated with the drugs for 6 h; this incubation time was previously shown to represent the optimal challenge period to induce PPARγ protein expression [8, 12, 14].

**PPARγ mRNA evaluation and real-time PCR**

Total RNA was extracted with the GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich), according to the manufacturer’s instructions. The amount and purity of total RNA were spectrophotometrically quantified by measuring the optical density at 260 and 280 nm. cDNA
synthesis was performed using a high-capacity cDNA reverse transcription kit (Applied Biosystems, USA) according to the manufacturer’s instructions. Real-time PCR was carried out in a volume of 20 μl per well in a 96-well optical reaction plate (Applied Biosystems) containing 1 μl of TaqMan Expression Assay (PPAR-γ), 5 μl of RNase-free water, 10 μl of TaqMan Universal PCR MasterMix (2 ×) (without AmpErase UNG) and 4 μl of cDNA template, as described [12]. The plate was run on the 7000 ABI Prism system (Applied Biosystems). To compensate for variations in cDNA concentrations and PCR efficiency between tubes, an endogenous gene control (β-glucuronidase) was included for each sample and used for normalization; results were analysed by the comparative cycle threshold method, as described [12].

Evaluation of MMP-9 activity

We used gelatin zymography to detect changes in MMP-9 activity in human monocytes and MDMs of RA patients and healthy donors. Cells (1 × 10⁶) were incubated for 6 h in RPMI 1640 medium; the supernatants were then mixed with Laemmll sample buffer (Bio-Rad) in a ratio 2:1 and analysed by electrophoresis with a 10% Novex zymogram gel (Invitrogen). The gel was developed according to the manufacturer’s instructions, with the Colloidal Blue Staining Kit (Invitrogen), and analysed by densitometer in non-saturating conditions (Versadoc). We also evaluated the ability of 15d-PGJ₂, MTX and MP to affect MMP-9 activity in monocytes/MDMs. In these experiments, cells (isolated from healthy donors) were pre-treated with the drugs for 1 h and then stimulated by lipopolysaccharide (LPS) 100 ng/ml for 6 h, which is the same time as for the determination of PPAR-γ protein expression.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5. Data are presented as mean (S.E.M.) of ‘n’ independent experiments on monocytes/macrophages from different patients or healthy donors, except otherwise stated. Spearman’s correlation coefficient was used to evaluate possible associations between variables that were not normally distributed, the following categories being used to express r values: negligible correlation (0–0.2), weak correlation (0.21–0.4), moderate correlation (0.41–0.7), high correlation (0.71–0.9), very high correlation (>0.9). Differences between groups were analysed using a non-parametric Mann–Whitney test. P < 0.05 was considered statistically significant.

Results

Characteristics of the patients enrolled in the study

The baseline demographic and clinical data for the 30 RA outpatients (20 females and 10 males) enrolled in this study are shown in Table 1. All patients were on current DMARD therapy: 22 receiving weekly MTX, 10 patients receiving other different DMARDs, in single or combination therapy. Twenty patients were also receiving low-dose oral CsSs (16 patients assumed prednisone and 4 patients had MP), with a mean prednisone dosage of 8.4 ± 5.6 mg daily. RA patients treated with monoclonal antibodies or TNF-α inhibitors and/or smoker patients were excluded from this study. Mean values for CRP, ESR, RF positivity and ACPA are presented in Table 1. The DAS-28 score was 0.63–5.97, with a mean value of 3 (1) (Table 1). Also 15 non-smoker healthy donors (5 males and 10 females) were enrolled in the study; they were age-matched to RA patients, had no history of inflammatory/immune or other chronic diseases and were drug-free (including paracetamol or aspirin) at the time of the study.

Expression of PPAR-γ protein and mRNA in monocytes/macrophages

As depicted in Fig. 1A, monocytes and MDMs obtained from RA patients present a significantly enhanced constitutive expression of PPAR-γ protein as compared with healthy donors (PPAR-γ/β-actin ratio = 0.21 ± 0.02 and 0.47 ± 0.05 in monocytes from 15 healthy donors and 30 RA patients, respectively; P < 0.001). Similar results are obtained in MDMs, with a PPAR-γ/β-actin ratio of 0.67 ± 0.08 and 1.8 ± 0.19 in healthy donors (n = 15) and RA patients (n = 30), respectively (Fig. 1A; P < 0.001). In keeping with previous observations [8, 12, 14], MDMs from both healthy donors and RA patients present a constitutively higher (P < 0.001) PPAR-γ expression than monocytes. Representative western blots for PPAR-γ protein expression are shown in Fig. 1B. In RA patients, PPAR-γ mRNA levels are increased (4-fold in monocytes and about 3-fold in MDMs) compared with healthy donors (Fig. 1C). As reported in Fig. 2, a significant weak inverse correlation between PPAR-γ protein expression and disease activity (evaluated by the DAS-28 score) was found in both monocytes (r = 0.4; P < 0.05) and MDMs (r = 0.31; P < 0.05). Due to the limited number of patients enrolled and their individual DAS-28 score, we pooled the data into two series, DAS-28 score = 3.2 representing the boundary line. As shown in Fig. 3A, monocytes and MDMs from patients with low disease activity (DAS-28 < 3.2) present a significantly higher PPAR-γ protein expression than healthy donors (P < 0.001) and RA patients with moderate-high (DAS-28 > 3.2) disease activity (P < 0.01) (Fig. 3A). In patients with DAS-28 > 3.2, PPAR-γ protein expression is higher than healthy donors for MDMs (P < 0.01) but not for monocytes (Fig. 3A). By evaluating PPAR-γ mRNA levels in monocytes/macrophages, we found a significant increase (P < 0.001 vs healthy donors in monocytes, and P < 0.05 in MDM) in all the RA patients, with no statistically significant difference according to the disease severity (Fig. 3B).

MMP-9 activity in monocytes/macrophages

As is known, MMP-9 activity is critical for RA progression and is inhibited by PPAR-γ agonists [7, 19–20]. In monocytes/MDMs from RA patients, the basal MMP-9 activity is significantly higher (P < 0.001 in monocytes, P < 0.01 in MDMs) than in healthy volunteers (Fig. 4A); representative gelatin zymographies are provided in Fig. 4B.
**Fig. 1** Enhanced PPARγ expression in monocytes and MDMs from RA patients.

(A) PPARγ protein expression in healthy donors (n = 15) and RA patients (n = 30). Semi-quantitative evaluation of PPARγ protein was performed by calculating the ratio between its total expression and β-actin. ***P < 0.001 vs healthy donors; ****P < 0.001 vs MDMs. (B) Constitutive expression of PPARγ protein. Representative blots from three healthy donors and eight RA patients. (C) PPARγ mRNA fold increase in cells from healthy donors (n = 9) and RA patients (n = 15 for monocytes; n = 14 for MDMs). Data are mean (S.E.M.); *P < 0.05 vs healthy donors; ***P < 0.001 vs healthy donors.

**TABLE 1** Demographic and clinical features of the studied population

<table>
<thead>
<tr>
<th></th>
<th>RA patients (n = 30)</th>
<th>Controls (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (s.d.), years</td>
<td>60.1 (14.2)</td>
<td>59.8 (7.3)</td>
</tr>
<tr>
<td>Female sex, n (%)</td>
<td>20 (66.7)</td>
<td>10 (66.7)</td>
</tr>
<tr>
<td>Disease duration, mean (s.d.), months</td>
<td>71.1 (68)</td>
<td>-</td>
</tr>
<tr>
<td>ESR, mean (s.d.), mm/h</td>
<td>19.4 (14)</td>
<td>-</td>
</tr>
<tr>
<td>CRP, mean (s.d.), mg/dl</td>
<td>0.6 (0.5)</td>
<td>-</td>
</tr>
<tr>
<td>RF positivity, n (%)</td>
<td>22 (73.3)</td>
<td>-</td>
</tr>
<tr>
<td>ACPA, n (%)</td>
<td>19 (63.3)</td>
<td>-</td>
</tr>
<tr>
<td>DAS-28 score, mean (s.d.)</td>
<td>3 (1)</td>
<td>-</td>
</tr>
<tr>
<td>Prednisone treatment, n (%)</td>
<td>20 (66.7)</td>
<td>-</td>
</tr>
<tr>
<td>Dosage, mean (s.d.), mg/day</td>
<td>8.4 (5.6)</td>
<td>-</td>
</tr>
<tr>
<td>DMARDs, n (%)</td>
<td>30 (100)</td>
<td>-</td>
</tr>
<tr>
<td>MTX treatment, n (%)</td>
<td>22 (73.3)</td>
<td>-</td>
</tr>
<tr>
<td>Other DMARDs (SSZ, HCQ, LEF), n (%)</td>
<td>10 (33.3)</td>
<td>-</td>
</tr>
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Some RA patients are treated with two or more drugs. -: not determined.
Interestingly, a positive moderate correlation ($r = 0.60; P < 0.05$) between MPP-9 activity and DAS-28 score was observed in both monocytes and MDMs (Fig. 4C). Therefore monocytes/MDMs isolated from patients with DAS-28 $>3.2$ present the highest gelatinolytic activity [17527 ± 2629 optical density (OD) in monocytes, $n = 8$; 36410 ± 8800 OD in MDMs, $n = 6$], with statistical significance ($P < 0.001$) vs healthy donors (2069 ± 563 OD in monocytes, $n = 6$; 3060 ± 640 OD in MDMs, $n = 6$) and vs RA patients with a DAS-28 score $<3.2$ (6877 ± 1253 OD in monocytes, $n = 10$; 11948 ± 2635 OD in MDMs, $n = 9$; $P < 0.01$ for both cell types).

Effects of MP and MTX on monocytes/macrophages from healthy donors

Because all the patients enrolled in this pilot study were treated with the conventional RA therapy that combines DMARDs and, most of the time, CSs (Table 1), we also evaluated the in vitro ability of MP and MTX to directly affect PPAR$\gamma$ expression and MPP-9 activity in cells from healthy donors, using the PPAR$\gamma$ agonist 15d-PGJ $10 \mu\text{M}$ as the positive control [8, 12]. Both drugs were used at concentrations that can be found in RA patients, MTX was used at $1 \mu\text{M}$, since Godfrey et al. [21], in a large pharmacokinetic study in RA patients, reported serum MTX concentrations of $0.3-1.5 \mu\text{M}$. Moreover, $C_{\text{max}}$ values of $1.1 \mu\text{M}$ for MTX and $180 \text{ng/ml}$ for MP are also reported [22]. As shown in Fig. 5A, MTX (used at $1 \mu\text{M}$) and MP (used at $180 \text{ng/ml}$, that is, $4.8 \times 10^{-7}\text{M}$) induce PPAR$\gamma$ protein expression in both monocytes and MDMs; the effects elicited by 15d-PGJ are shown for comparison (Fig. 5A) and representative western blots are provided (Fig. 5B). Moreover, in the range $10^{-10}-10^{-5}\text{M}$, MP induces concentration-dependent PPAR$\gamma$ protein expression ($E_{50} = 84 \text{nM}$) (data not shown). Because MMP-9 exerts a crucial role in RA progression and joint destruction [1], inhibition of the gelatinolytic activity can contribute to the overall clinical efficacy of a given drug in RA therapy. In human monocytes and MDMs, LPS (100 ng/ml, 6 h) stimulates about 2-fold MPP-9 activity (5892 ± 968 OD in monocytes, $n = 4, P < 0.01$ vs control; and 9753 ± 310 OD in MDMs, $n = 4, P < 0.01$ vs control; data not shown). As shown in Fig. 6, 15d-PGJ, MTX and MP potently inhibit LPS-induced MPP-9 activity, the endogenous PPAR$\gamma$ ligand and MTX being more effective than MP ($P < 0.001$; Fig. 6A) in monocytes. In contrast, the three drugs similarly inhibited LPS-induced MPP-9 activity in MDMs (Fig. 6A); representative gelatin zymographies are provided in Fig. 6B.

### Discussion

This study indicates that monocytes/MDMs prepared from RA patients under pharmacological treatment (MTX and CSs, mainly) present an enhanced PPAR$\gamma$ expression (both protein and mRNA) as compared with cells of healthy donors, the extent of protein expression being inversely associated with disease activity. PPAR$\gamma$ over-expression in monocytes/macrophages from RA patients not only confirms RA as a systemic inflammatory disease, but also suggests that at the joint level, cells can be recruited from a pool of pre-activated peripheral monocytes.

Previous findings demonstrated increased PPAR$\gamma$ mRNA levels in a model of adjuvant-induced arthritis [5]. Moreover, Jiang et al. [23], by evaluating PPAR$\gamma$ gene expression in bone marrow cells obtained from patients with traumatic femoral neck fracture, OA or RA, observed that RA patients (but not OA patients) had significantly higher PPAR$\gamma$ mRNA levels than fractured subjects [23]. Increased PPAR$\gamma$ mRNA levels were also detected in macrophages from patients with active SLE as compared with patients with inactive SLE or infectious diseases and healthy donors [24], and we previously reported that monocytes/macrophages from patients with coronary artery disease present a constitutively enhanced PPAR$\gamma$ expression as compared with healthy subjects [12, 14].

The fact that PPAR$\gamma$ protein and/or mRNA might be increased in inflammatory/immune diseases with a relevant involvement of monocytes/macrophages is not surprising because PPAR$\gamma$ is a key modulator of macrophage differentiation [8, 25-26] and participates in different inflammatory and autoimmune disorders.
In this regard, a recent paper demonstrates that synovial tissues obtained from arthritis-susceptible and highly erosion-prone (DA rats) or arthritis-resistant (DA.ACI.Cia25 rats) animals 21 days after induction of arthritis present different levels of PPARγ expression, cytokine release and disease severity [29]. Indeed, reduced expression of inflammatory cytokines and proteases implicated in joint damage and erosions is detected in arthritis-resistant rats as compared with DA rats, and is matched by a 5-fold increased expression of PPARγ in resistant animals [29]. Analysis of gene expression also indicates a reduced number of infiltrating macrophages in resistant animals, with both Cd163 and Cd68 genes (which are relevant for M2 and M1 macrophages, respectively) being expressed more in arthritis-susceptible rats [29]. Our results are in keeping with these animal data because we measure significantly higher PPARγ protein expression in patients with lower disease activity.

It has also been suggested that M1 (the classically activated, inflammatory macrophages that secrete higher levels of pro-inflammatory genes and probably contribute to inflammation) and M2 macrophages (the less inflammatory, alternatively activated macrophages) can switch from one phenotype to the other [30]. Therefore the local environment created by the activation of PPARγ might induce a switch from M1- towards M2-activated macrophages, thus contributing to the anti-inflammatory effect. Interestingly, PPARγ is required to promote the M2 phenotype [31] and rosiglitazone has been shown to up-regulate markers
(e.g. arginase 1, IL-10) characteristic of the M2 phenotype [32]. In addition, glucocorticoids represent another stimulus that favours the M2 phenotype [33], and this switching could be relevant for their anti-inflammatory action at the macrophage level [34].

Although most of our RA patients were treated with oral prednisone, as a prototype of CSs for in vitro studies we used methylprednisolone because its characteristics—bioactivity per se and higher liposolubility—make it more suitable for a 6-h challenge, for the evaluation of PPARγ protein expression and MMP-9 activity. We show that besides reducing MMP-9 activity (see below), MTX and MP enhance in vitro PPARγ protein expression in monocytes and MDMs, as the endogenous ligand 15d-PGJ does. This is in accordance with previous reports demonstrating that indomethacin and other selected NSAIDs, telmisartan and some statins act as PPARγ agonists at relatively high concentrations, this property contributing to their overall therapeutic activity [6, 34–39]. Given that in our in vitro experiments MTX and MP increase PPARγ expression at therapeutic concentrations, it is tempting to speculate that this activity may contribute to their clinical efficacy in RA. In this context, it is worth noting that RA patients with a DAS-28 score <3.2, which reasonably identifies those patients in which MTX and CSs are effective in controlling disease severity, express significantly higher PPARγ protein levels than patients with DAS-28 score >3.2, which could indicate inadequately responding patients. Interestingly, the highest PPARγ protein expression (PPARγ/β-actin ratio = 0.74 ± 0.1 in monocytes and 2.6 ± 0.5 in MDM; data not shown) is documented in the seven RA patients with a score between 2 and <2.6, which, in keeping with Felson et al. [40], represents minimal disease activity rather than remission. It should be noted that PPARγ mRNA levels, even if significantly enhanced as compared with healthy donors, are similar in both DAS-28 cohorts. Although we have no definite explanation, this is in accordance with our previous results in patients with coronary artery disease [12].

These findings support the role of PPARγ in the pathophysiology of RA and suggest that DMARD-induced over-expression of PPARγ protein may contribute to their anti-rheumatic effects. In fact, induction of protective

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**Fig. 4** Enhanced MMP-9 activity in monocytes and MDMs from RA patients.

(A) MMP-9 activity in cells from RA patients (n = 18 for monocytes; n = 15 for MDMs) as compared with healthy donors (n = 6). ***P < 0.001 vs healthy donors; **P < 0.01 vs healthy donors. (B) Representative gelatin zymographies for MMP-9 activity in two healthy donors and three RA patients. (C) Correlation between MMP-9 activity and DAS-28 score in cells from RA patients.
receptors could be a relatively common downstream feature of DMARDs. As an example, MTX has been shown to release adenosine both in vivo and in vitro and to exert part of its effects via the anti-inflammatory adenosine A$_{2A}$ or A$_{3}$ receptors [41/42]. The formyl peptide receptor (FPR) type 2, first described in human monocytes [43], is another anti-inflammatory G-protein-coupled receptor that transduces signals from lipoxin A$_4$ and annexin A1 (AnxA1) [44]. Dexamethasone, which releases AnxA1 from neutrophils in a few minutes, inhibits neutrophil migration in different models of acute inflammation, but its effects are markedly reduced in Fpr2$^{-/-}$ mice [44]. Interestingly, Dufton et al. [44] observed that Fpr2$^{-/-}$ mice present an increased sensitivity to arthrogenic serum and also proved a marked up-regulation of fpr2 gene expression in the inflamed joints during the development of arthritic response.

Because proteinase activity of synovium is stronger in RA than in osteoarthritis and PPAR$_{\gamma}$ ligands inhibit MMP-9 secretion [19-20], we also measured MMP-9 activity in monocytes/macrophages. Our results confirm that MMP-9 activity is potently increased in RA patients as compared with healthy donors and that 15d-PGJ, the endogenous PPAR$_{\gamma}$ agonist, inhibits by 60-70% LPS-induced MMP-9 activity in cells from healthy donors. MTX and MP, evaluated in vitro at conceivable therapeutic concentrations (1 $\mu$M and 180 ng/ml, respectively), also inhibit LPS-induced MMP-9 activity; MTX demonstrates the same inhibitory effect as 15d-PGJ, whereas in human monocytes MP exerts a significantly lower inhibition (about 25%). In our opinion, this can be largely explained by the short period (1h pre-incubation with drugs + 6h LPS challenge) of the experiment and does not imply a reduced clinical effect of the CS.

Even more relevant is the positive correlation between MMP-9 activity and disease severity: MMP-9 activity in cells from patients with moderate/high disease (DAS-28 $>3.2$) was significantly up-regulated as compared with patients with DAS-28 $<3.2$ (about 3-fold increase; $P < 0.01$) and healthy donors (about 8-fold increase in monocytes and 12-fold increase in MDMs; $P < 0.001$).

Taken together, the key findings of enhanced PPAR$_{\gamma}$ expression and concomitant reduction in MMP-9 activity in cells from RA patients with less severe disease suggest PPAR$_{\gamma}$ expression in monocytes/macrophages as a possible indicator of disease activity and, consequently, successful RA therapy. On this regard, a recent small clinical trial in diabetic RA patients showed that the concomitant use of pioglitazone and MTX for 3 months, besides lowering blood glucose levels, significantly improved many RA markers, including swollen joint count, tender joint count and DAS-28 score [45].

Some limitations of this study warrant specific comments. First, all the RA patients evaluated were in stable disease conditions, most of them presenting low CRP and
ESR values, as well as not so severe DAS-28 score. Secondly, the number of patients enrolled in this study was too low to allow a detailed sub-group analysis; that is why we used a dichotomous (<3.2 or >3.2) DAS-28 score.

However, based on the experimental findings of this pilot study, it seems conceivable to suggest PPARγ expression in human monocytes/macrophages as an indicator of disease activity and/or therapy efficacy. However, further prospective studies with a larger cohort of patients are needed to confirm these preliminary results.

**Rheumatology key messages**

- Monocytes/macrophages from RA patients present higher PPARγ expression and MMP-9 activity than those from healthy donors.
- PPARγ protein expression and MMP-9 activity in RA patients are related to DAS-28 score.
- We suggest PPARγ expression as a relevant indicator of disease activity and therapy efficacy in RA.

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