Macrophage migration inhibitory factor in rheumatoid arthritis: clinical correlations

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Abstract

Objective. Cytokines play an important role in the pathology of rheumatoid arthritis (RA). Macrophage migration inhibitory factor (MIF) is a cytokine with a broad spectrum of actions, including induction of monocyte tumour necrosis factor \( \alpha \) (TNF-\( \alpha \)). Evidence of the expression and proinflammatory activity of MIF has recently been demonstrated in RA synovium and in animal models of RA. We wished to assess the relationship between MIF expression in synovium and clinical disease.

Methods. Computer-assisted analysis of the cytokine content of arthroscopically obtained biopsies of RA synovium, using paired samples from eight patients with active and inactive untreated disease, was compared with documented clinical parameters.

Results. Synovial MIF immunostaining correlated strongly with disease activity as measured by CRP concentration. Reductions in clinical disease parameters, including CRP, tender and swollen joint counts, were accompanied by significant reductions in synovial MIF. Synovial TNF-\( \alpha \), transforming growth factor \( \beta \) (TGF-\( \beta \)) and interleukin (IL) 10 also showed a significant reduction in association with reduced disease activity, while IL-1\( \beta \) and IL-1 receptor agonist did not.

Conclusion. The correlation of synovial MIF with disease activity corroborates existing evidence of the role of this cytokine in RA. The demonstration that only MIF and TNF-\( \alpha \) show significant variation in synovial cytokine content with clinical remission suggests that MIF is an important member of the cytokine hierarchy in RA.

Key words: Macrophage, Fibroblast, Glucocorticoids, Synovial, Cytokine, Macrophage migration inhibitory factor, Rheumatoid arthritis.

Macrophage migration inhibitory factor (MIF) is a cytokine with increasingly well recognized importance in the regulation of immune and inflammatory responses. Its original description focused on its ability to prevent the random migration of macrophages in culture, but since its cloning in the mid-1990s evidence of a much broader range of proinflammatory actions has emerged. MIF is released by activated T-lymphocytes and macrophages and up-regulates the proinflammatory activity of these cells [1–3]. Animal studies demonstrate a critical role of MIF in the development of both endotoxic shock and delayed-type hypersensitivity [4, 5], suggesting important functions in both the innate and the cognate immune response.

In human disease, important pathogenic contributions of MIF have been suggested by studies in acute pulmonary inflammation [6] and ocular inflammation [7]. Most recently, MIF has been described in human rheumatoid arthritis (RA) synovium [8]. Expressed by synovial macrophages and fibroblast-like synoviocytes (FLS), MIF is overexpressed in RA serum, synovial fluid and FLS compared with controls. Moreover, evidence of an upstream role for MIF in the RA cytokine cascade was provided by the demonstration that MIF derived from RA FLS induced the release of tumour necrosis factor \( \alpha \) (TNF-\( \alpha \)) by monocytes [8], and MIF has been shown to contribute to synoviocyte activation \textit{in vitro} [9]. Monoclonal antibodies to MIF have been shown to have profound inhibitory effects on joint inflammation in rodent models of RA [10, 11]. These data make it clear that MIF is an important cytokine in the RA lesion.

Evolving therapeutic strategies in RA target specific mediators of disease, such as TNF-\( \alpha \). These strategies...
are based on the assumption that reducing tissue levels of cytokines will lead to improvements in clinical parameters. Impressions of the clinical value of reducing synovial cytokine expression can also be suggested indirectly by studies on the variation in synovial cytokine expression with changes in clinical disease activity. In order to further explore the status of MIF in the hierarchy of synovial cytokines, we hypothesized that synovial MIF would vary with disease activity. We therefore examined cytokine expression in paired synovial biopsies obtained from patients in active and treated phases of RA. Our results demonstrate that, like TNF-α, synovial MIF varies significantly with disease activity in RA.

**Methods**

**Patients**

Synovial samples were obtained from individuals meeting the American College of Rheumatology criteria for the classification of RA [12], using an arthroscopic protocol as described previously [13]. Initial biopsies were obtained from patients presenting with active untreated disease, including active synovitis in the biopsied knee joint. Biopsies were obtained subsequently from the same patients’ knee when disease activity was reduced by conventional anti-rheumatic drug therapy, after 423 ± 97 days (mean ± s.e.m.). All repeat biopsies but one were from knees with no clinical signs of active synovitis. Treatment was not given according to fixed protocols, and included methotrexate, sulphasalazine, intramuscular gold salts and glucocorticoids. No patient received intra-articular corticosteroid in the study joint in the study interval. The study protocol was approved by institutional ethics boards and all subjects gave written informed consent.

**Clinical assessment**

All patients were assessed clinically by one investigator (MDS) using standard methods for acquiring tender and swollen joint counts and assessing the duration of early morning stiffness. CRP was measured by rate nephelometry.

**Synovium**

Specimens of synovium were obtained from eight patients under direct vision using a 2.7 mm needle arthroscope (Dyonics, Andover, MA, USA). Tissues were snap-frozen for subsequent immunohistochemistry, performed as published [8]. Briefly, sections were incubated with rabbit serum and then with primary mouse anti-human cytokine monoclonal antibody (mAb) or isotype control immunoglobulin G (IgG). Sections were stained with mAb directed at MIF, interleukin (IL)-1β, TNF-α, IL-10, transforming growth factor (TGF) β and IL-1 receptor antagonist (IL-1RA). Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in methanol and sections were then stained using rabbit anti-mouse IgG and peroxidase anti-peroxidase complex and diaminobenzidine tetrahydrochloride–H₂O₂ solution.

**Scoring of immunostained sections**

The immunostained sections were examined using a previously validated computer-assisted video image analysis system [14]. Briefly, the integrated optical density (IOD) was measured pixel by pixel in each synovial region; this represented the total amount of cytokine per region. The mean optical density (MOD) was then calculated from IOD/x, where x is the area examined per region in mm² and represents the amount of cytokine staining per unit area. At least two biopsies were obtained from each knee joint at each time point, and at least six randomly selected sections of synovium were analysed from each biopsy. All sections were measured by one blinded observer; the repeatability of measurements was within 10%.

**Statistical analysis**

All data are expressed as mean ± s.e.m. Statistical analysis was performed on a summary measure for each patient at each time point. The correlations between cytokine immunostaining and other variables were analysed using the Pearson coefficient. Paired Student’s t-tests were used for comparison of groups, making the assumption of a normal distribution. All data sets were tested for deviation from Gaussian distribution using the Kolmogorov–Smirnov test using Prism software (Graphpad, San Diego, CA, USA), and passed a test of normality. Exclusion of the single subject with synovitis at the time of the second biopsy did not alter the significance of any analysis.

**Results**

Clinical features of patients from whom synovium was obtained are shown in Table 1. Initial biopsies were obtained during a phase of active untreated disease, as determined by high values of serum CRP, tender joint count, swollen joint count and early morning stiffness.

Relationships between cytokine staining and clinical variables were sought in active disease samples. In

**Table 1. Markers of disease activity (mean ± s.e.m.) measured during active, untreated disease and after successful treatment with anti-rheumatic drugs**

<table>
<thead>
<tr>
<th></th>
<th>Active</th>
<th>Inactive</th>
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<tr>
<td>Age (yr)</td>
<td>69.5 ± 3.2</td>
<td>7.2 ± 2</td>
</tr>
<tr>
<td>Disease duration (yr)</td>
<td>90.5 ± 18.1</td>
<td>7.4 ± 6.4</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>217.5 ± 25.2</td>
<td>7.5 ± 3.7</td>
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<tr>
<td>Early morning stiffness (min)</td>
<td>203 ± 1.7</td>
<td>34 ± 1.6</td>
</tr>
<tr>
<td>Tender joint count</td>
<td>16 ± 2.2</td>
<td>25 ± 1.3</td>
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<tr>
<td>Swollen joint count</td>
<td>8.7 ± 0.4</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>VAS pain (0–10 cm)</td>
<td>9.0 ± 0.0</td>
<td>7.4 ± 0.5</td>
</tr>
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VAS, visual analogue scale.
active disease synovia, synovial lining layer MIF MOD (0.42 ± 0.04) was significantly higher than sublining layer MIF (0.35 ± 0.03, P < 0.0002). Synovial lining and sublining MIF were tightly correlated (r = 0.94, P < 0.001). Synovial lining MIF MOD was significantly correlated with serum CRP (r = 0.745, P = 0.03) and a significant negative correlation was observed with synovial IL-1RA (r = -0.878, P = 0.0023).

Repeat biopsies were obtained in a period of significantly reduced disease activity induced by drug treatment, as assessed by serum CRP, tender joint count, swollen joint count and early morning stiffness (Table 1). Differences in synovial cytokine content in synovia from initial biopsies taken during active disease were assessed using IOD in order to take into account changes in synovial cellularity. Compared with MIF staining in active disease, synovial lining layer MIF was significantly reduced (P = 0.031) (Fig. 3 and Table 2). Synovial lining TNF-α (P < 0.01), IL-10 (P < 0.05) and TGF-β (P < 0.05) were also significantly reduced in treated synovia (Table 2). No significant reductions in IL-1β or IL-1RA were observed, although the reduction in IL-1RA approached significance (Table 2).
Discussion

The cytokine profile of RA synovium is an area of intense research interest. Proinflammatory cytokines such as TNF-α and IL-1β are known to induce, in vitro, many of the mediators of inflammation and joint injury operative in RA. Moreover, discovery of the expression of these cytokines in RA synovium has led to the development of specific therapeutic strategies targeted at these molecules. To date, however, no such strategies have proved curative, and the search for other therapeutic targets in RA continues.

Many aspects of the function of MIF suggest it as an important cytokine in RA. Recombinant MIF is an inducer of monocyte/macrophage TNF-α and MIF also induces or enhances other aspects of monocyte/macrophage activity, including interferon-γ-induced nitric oxide production, intracellular killing and phagocytic function. Unlike other cytokines which activate monocyte/macrophages, MIF has also been demonstrated to be a crucial cofactor in T-cell activation [2] and is required for the expression of delayed-type hypersensitivity in vivo [5]. In addition, the recent report of the expression of MIF by endothelial cells suggests it has a uniquely broad range of activities within the immune/inflammatory response involved in RA pathology [15]. Recent experiments have supported an important role for MIF in RA. MIF is readily detected in RA synovial tissue samples, where it is expressed predominantly by monocyte/macrophages and fibroblast-like synoviocytes (FLS) [8]. The overexpression of MIF in RA synoviocytes, synovial fluid and serum compared with controls also supports its participation in the inflammatory response in RA. MIF mRNA is expressed constitutively and MIF protein is released constitutively by cultured RA FLS [8]. Moreover, we have shown that MIF released by RA FLS induces monocyte TNF-α, implicating MIF as a factor responsible for TNF-α production in RA [8]. Together with the potent suppressive effects of anti-MIF mAb in animal models, including adjuvant and collagen arthropitits [10, 11], these data strongly suggest that MIF is an upstream member of the RA cytokine cascade. This is further supported by the observation of reduced TNF-α production by monocytes from mice bearing a deletion of the MIF gene [16]. MIF has also been shown recently to induce key aspects of fibroblast-like synoviocyte activation, including the expression of phospholipase A2 and cyclooxygenase-2, and cell proliferation [9]. Moreover, we have recently shown 100% protection from mortality in adrenalectomized rats with adjuvant arthritis when treated with anti-MIF mAb, again consistent with an important role for this cytokine in systemic and local inflammation [17].

Considerably more research will be required in order to validate the importance of MIF in RA. One approach which can yield clues to the importance of cytokines or mediators is to examine relationships between their expression and variations in disease expression. Ideally, this would be pursued by examination of samples from patients with natural variation in disease activity, but ethical considerations prevent patients with active RA from going untreated. Currently, therefore, the most readily available method by which to assess variations in clinical disease and synovial mediators is to examine synovial tissue from patients with changes in disease activity induced with drug treatment.

The current data lend support to the idea that MIF is an important cytokine in RA. Synovial MIF was strongly correlated with an objective measure of disease activity—serum CRP concentration. Supporting the concept of the clinical relevance of this association was the observation that IL-1β and IL-1RA exhibited positive and negative correlations respectively with CRP in this group of patients. This is consistent with the pro- and anti-inflammatory actions of these cytokines. Moreover, significant reductions in synovial MIF content were observed in patients with inactive disease following conventional drug therapy. Other cytokines, including TNF-α, IL-10 and TGF-β, also varied significantly with this change in clinical status. The small sample size in the present study may have contributed to the failure to detect significant changes in IL-1β and IL-1RA. Nonetheless, inclusion of MIF in the group of cytokines significantly influenced by treatment-induced reductions in disease activity suggests that it may be an important cytokine in RA. The association of reduced synovial MIF with improved disease suggests that lowering synovial MIF by specific targeting approaches, for example with antibody therapy, may be effective in RA.

The lack of coordinate variation in synovial expression of all cytokines in the current study is of interest. Recently, evidence has emerged suggesting independent contributions of cytokines to joint inflammation and injury. In particular, studies on the roles of IL-1 and TNF-α in animal models of RA suggest that suppression of TNF-α alone, while significantly inhibiting inflammation, has minimal effects on cartilage degradation, while the reverse is true for IL-1β [18]. In the present study, only MIF exhibited both a significant correlation with disease activity and significant reduction in inactive disease. Reductions in IL-10 and TGF-β may be consistent with the induction of synthesis of these notionally anti-inflammatory cytokines by ‘upstream’ cytokines, including TNF-α. The contribution of MIF to the regulation of IL-10 and TGF-β is less well understood, although both may antagonize the actions of MIF on macrophage activation as part of their spectrum of counter-regulatory functions [19].

An important aspect of MIF biology is its relationship with glucocorticoids. The synthesis and release of most proinflammatory cytokines is inhibited by glucocorticoids. We recently showed that, in RA FLS, while high concentrations of glucocorticoid were indeed suppressive, low concentrations in the physiological range up-regulated MIF mRNA and protein [8]. This is consistent with previous evidence for glucocorticoid inductibility of MIF in macrophages [20]. Moreover, while induced by glucocorticoids, MIF is capable of...
reversing glucocorticoid inhibitory effects on monocyte/macrophage and T-cell function [2, 20], suggesting a unique counter-regulatory role for MIF in relation to the suppressive effects of endogenous glucocorticoids on the immune system. No patient in this study received glucocorticoids without additional drug treatment, and a direct interpretation of the effects of glucocorticoids on synovial MIF is therefore impossible.

In summary, our observation of a relationship between synovial expression of MIF and disease activity supports the hypothesis that MIF is an important contributor to the clinical expression of RA. These data further support the view that MIF is a potential therapeutic target in RA.

References


