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Metastasis-inducing S100A4 protein is associated with the disease activity of rheumatoid arthritis

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Objectives. To evaluate the association between metastasis-inducing protein S100A4 and disease activity in patients with RA, and to demonstrate the effect of TNF-α blocking therapy on plasma levels of S100A4 in these patients.
Methods. Plasma levels of the S100A4 protein were analysed in 40 anti-TNF-α naive patients with active RA. Of the 40 patients, 25 were treated with adalimumab and monitored over time. The conformational form of S100A4 was analysed using size-exclusion gel chromatography. TNF-α mRNA expression and protein synthesis were analysed by RT–PCR and ELISA, respectively.
Results. Baseline levels of S100A4 were significantly correlated with disease activity in RA patients ($r = 0.41$; $P < 0.01$). After 12 weeks of treatment with adalimumab, there was an obvious shift in the conformations of S100A4 from the multimeric to the dimeric forms, whereas the total levels of the S100A4 protein remained unchanged. This suggests that the bioactive (multimer) S100A4 may decline in response to successful treatment with adalimumab. In addition, we showed significant up-regulation of TNF-α mRNA ($P < 0.01$), and protein release to the cell culture medium of monocytes stimulated with the S100A4 multimer compared with those treated with the dimer and to the unstimulated monocytes ($P < 0.001$).
Conclusions. This is the first study to show that the levels of the S100A4 protein are correlated with RA disease activity. Furthermore, only the bioactive form, but not the total amount of S100A4, decreases after successful TNF-α blocking therapy in patients with RA. These data support an important role for the S100A4 multimer in the pathogenesis of RA.

Key words: S100A4 protein, Rheumatoid arthritis, Inflammation, TNF-α inhibitor.

Introduction

RA is a systemic, autoimmune, inflammatory disease characterized by persistent inflammation of synovial joints and progressive joint destruction. The exact aetiopathogenesis of RA is still uncertain. Nevertheless, several different cell types, such as T cells, B cells, macrophages and synovial fibroblasts have been shown to produce a wide range of cytokines and inflammatory mediators that contribute to the tissue-destructive process of the disease [1–3]. TNF-α, which is secreted by most of these cells, is one of the central drivers of RA's pathology [4, 5]. As a result, TNF-α inhibitors have been introduced as an effective treatment for patients with active RA [6, 7].

The S100 protein family represents the largest group of calcium-binding proteins, and it is known to be involved in diverse biological regulatory activities [8]. The best-known members of the family, S100A8/A9 and S100A12, have been localized to inflammation sites and proposed to act as alarmins—endogenous molecules that signal tissue and cellular damage [9, 10]. S100 alarmins can be released, exert cytokine-like activities and trigger a variety of intracellular signalling pathways [9, 10]. Both S100A8/A9 and S100A12 have been previously found to be up-regulated in inflamed synovial tissue, SF and in circulating blood of patients with RA. In addition, a tight association between these proteins and the activity of the disease has been recently demonstrated in patients with several arthritides [11–14]. Furthermore, the levels of both alarmins decrease in response to different anti-inflammatory therapies and become up-regulated even weeks before the clinical appearance of relapse in patients with previously well-controlled disease [15, 16]. In addition, the decline in both S100 proteins in the synovial tissue and in the bloodstream has also been demonstrated in RA patients who were treated with the TNF-α inhibitors, infliximab or etanercept [14, 16, 17].

We have recently reported that another member of the S100 family, S100A4, is significantly up-regulated in activated synovial fibroblasts, hyperplastic synovial tissue and in SF and plasma of RA patients [18–21]. S100A4, also known as calvasculin, fibroblast-specific protein (FspI) or metastasin (Mts1), was first described 20 years ago as a metastasis-specific gene product [22]. Since then, it has become evident that S100A4 contributes not only to the regulation of tumour progression and metastasis (for review see Helfman *et al.* [23]), but that it may also participate in the process of inflammation and cartilage destruction [24, 25]. Therefore, the aim of this study was to evaluate the association between the S100A4 protein and disease activity, and to measure the effect of the TNF-α inhibitor adalimumab on the plasma levels of S100A4 protein in patients with active RA.

Methods

Patients

Forty patients with active RA were enrolled in this study [34 females and 6 males; mean (S.D.) age 50.6 ± 13.3 years]. All patients fulfilled the revised criteria of the ACR for the diagnosis of RA [26]. Twenty-five of these patients (21 females and 4 males; mean age 43.7 ± 10.3 years) were treated with the anti-TNF-α agent adalimumab and then followed for the following 12 weeks. Adalimumab was administrated subcutaneously in a standard dose of 40 mg every other week. The mean dose of MTX and glucocorticoids did not differ during the treatment. The disease activity of RA patients was assessed according to the 28 joint count disease activity score (DAS28) using the number of swollen and tender joints, ESR and patient's global visual analogue scale (VAS) [27]. Baseline characteristics of the patients are provided in Table 1. The study was approved by the

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local ethics committee, and informed consent was obtained from all patients.

Laboratory analyses

Blood samples were collected from all patients at baseline and, additionally, from the adalimumab group at Weeks 6 and 12 after initiation of therapy. Samples were immediately centrifuged and stored at −20°C until analysis. CRP was analysed by latex immunoturbidimetric assay according to the manufacturer's protocol (Olympus, Prague, Czech Republic). Plasma S100A4 concentration was measured by a sandwich ELISA, as previously described [21].

Size-exclusion chromatography (SEC) was carried out using a Superdex 200 PC 3.2/30 column (Amersham Biosciences, Hillerod, Denmark) with buffer A (50 mM Tris–HCl pH 7.4, 144 mM NaCl) as the mobile phase. The column was calibrated using a gel filtration standard (Bio-Rad Laboratories, Prague, Czech Republic) ranging from 1.35 to 670 kDa. The mixture included thyroglobulin (670 kDa), bovine γ-globulin (158 kDa), chicken ovalbumin (44 kDa), equine myoglobin (17 kDa) and vitamin B12 (1.35 kDa). A plasma volume of 20 μl was applied to the column. The chromatography profile was monitored at 215 nm. Fractions of 50 μl were collected, snap-frozen in liquid nitrogen and stored at −80°C.

Stimulation of monocytes by S100A4

Human peripheral blood mononuclear cells were isolated from the peripheral blood of healthy volunteers ($n=4$) by Ficoll-Plaque density gradient centrifugation. $CD14^+$ monocytes were selected positively using anti-CD14 microbeads (Miltenyi Biotec, Bergisch Gladback, Germany). Monocytes were plated at 5.0×10^5 cells (confluent cells condition) per 2 ml of advanced RPMI medium 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with L-glutamine (Gibco, Carlsbad, CA, USA) in 35-mm diameter culture plates. After plating, monocytes were incubated for 30 min at 37°C with 5% CO_2 in a humidified atmosphere. Non-adherent cells were eliminated by sterile PBS wash. Adherent cells were treated with a S100A4 dimer (1 μg/ml) and multimer (1 μg/ml) for 6 and 24 h. The S100A4 proteins were obtained from recombinant His6-tagged protein by gel filtration [28]. The dose and time course were selected in accordance with our previous experiments [19, 21]. The incubation was terminated after 6 h and cells were lysed by RLT buffer (Qiagen, Hilden, Germany) and stored in −80°C until RNA isolation. Cell culture supernatants were collected after 24 h of exposure to S100A4 and stored in −20°C.

RT–PCR analysis

Total RNA from monocytes was extracted using the MagNA Pure Compact RNA Isolation kit for MagNA Pure Compact Instrument (Roche Diagnostics, Mannheim, Germany). Complementary DNA was obtained by reverse transcription using RevertAid First Strand cDNA Synthesis Kit (Fermentas Life Sciences, Vilnius, Lithuania). PCR was performed according to a standard protocol. Pre-developed, commercially available primers (Applied Biosystems, Prague, Czech Republic) were used to detect TNF-α mRNA. The 18S rRNA was used to normalize the results. Data were analysed using the comparative threshold cycle (C_t) method for relative quantification, and the C_t for 18S samples was subtracted from the C_t of the studied genes, giving the dC_t values. Real-time PCR was performed using the 7900HT Fast Real-Time PCR System (Applied Biosystems).

Table 1. Baseline characteristics of the patients with RA

	Subgroup of RA patients treated with anti-TNF-α	All RA patients—anti-TNF-α naive
Number	25	40
Gender (female/male)	21/4	34/6
Age, years	43.7 (10.3)	50.6 (13.3)
CRP, mg/l	38 (28.9)	29.9 (26.5)
DAS28 score	6.2 (0.7)	5.8 (1.2)
Drugs (MTX/GC)	19/16	28/27
ESR, mm/first h	39.8 (20.1)	34.3 (20.1)
S100A4, ng/ml	466.5 (306.1)	592 (458.3)

S100A4 concentrations are expressed as mean (S.E.M.). GC: glucocorticoids; DAS: disease activity score; CRP: C-reactive protein Other values as mean (S.D.).

ELISA

The levels of TNF-α were measured in the cell culture supernatants using an ELISA kit according to the manufacturer's protocol (Ray Biotech, Norcross, GA, USA). The analysis was performed using a Sunrise ELISA reader (Tecan, Salzburg, Austria) at a wavelength of 450 nm.

Statistical analyses

S100A4 protein concentrations were expressed as mean (S.E.M.). Spearman's rank correlation was used to correlate any two variables. The Friedman analysis of variance test for repeated measures and Mann–Whitney U-test for comparisons between two measures were performed. For all statistical evaluations, P-values <0.05 were considered as statistically significant.

Results

S100A4 protein and disease activity

Disease activity decreased significantly after treatment with adalimumab [mean (S.D.) DAS28: 6.2 (0.7) (baseline) *vs* 3.8 (1.0) (Week 6) *vs* 3.3 (0.9) (Week 12); $P<0.01$]. The treatment was clinically effective in 23 out of 25 RA patients (δ DAS28: $\geqslant1.2$). The baseline level of S100A4 in plasma correlated strongly with the baseline DAS28 ($r=0.41$; $P<0.01$) (Fig. 1). However, no correlations were observed between the S100A4 protein and the DAS28 at Weeks 6 and 12 after the initiation of therapy, as well as between the baseline S100A4 and the change in DAS28 between baseline and Week 12 ($r=0.23$; $P=0.26$). Levels of S100A4 protein did not correlate with the baseline levels of CRP ($r=-0.17$; $P=0.29$), IgM RF ($r=0.02$; $P=0.9$) or with anti-citrullinated protein/peptide antibodies (ACPAs) ($r=0.09$; $P=0.56$). Likewise, these variables did not correlate with S100A4 levels after the treatment (data not shown). Results were indicative of a relationship between S100A4 protein and the inflammatory activity, rather than with the autoimmune response of the disease.

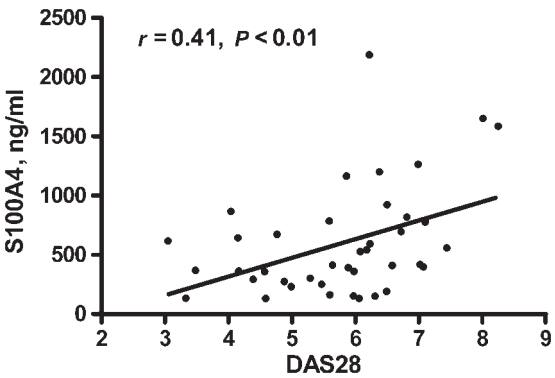

Fig. 1. Relationship between the baseline disease activity and plasma levels of the metastasis-inducing protein S100A4 in patients with RA.

The effect of TNF-α inhibitor adalimumab on the plasma S100A4 protein

S100A4 values in plasma of healthy individuals are normally in the range of 50–300 (mean 80) ng/ml, as reported in our previous study [21]. As shown in Fig. 2A, the total plasma levels of S100A4 were significantly higher, but not significantly affected by adalimumab treatment [mean (S.E.M.) of baseline *vs* Week 6 *vs* Week 12: 466.4 (66.5) *vs* 522.1 (58.4) *vs* 535.1 (96.8) ng/ml] in patients with RA. Plasma samples from eight randomly selected patients were subjected to SEC, and six of them demonstrated a tendency to shift from the high to the low conformational form after 12 weeks of adalimumab therapy. Two representative profiles of the SEC are shown in Fig. 2B and C. One of the two samples showing no clear conformational shift in S100A4 belonged to a patient who failed to achieve a clinically effective response (Fig. 2D). Patients responding to the treatment with adalimumab showed a clear shift in the conformations of S100A4 from the multimeric and oligomeric forms to the dimeric forms. In contrast, no such clear shift was observed in a patient who experienced an inadequate response to the treatment with adalimumab. This patient presented, instead, a tendency for an increase in some multimeric and oligomeric S100A4 forms. Therefore, it can be suggested that successful treatment of RA patients yields the prompt conformational modifications of the bioactive S100A4 multimer towards the less bioactive S100A4 dimer.

S100A4 multimer up-regulates TNF-α in human monocytes

To confirm the bioactivity of the S100A4 multimer, we stimulated peripheral blood monocytes with the S100A4 multimer and dimer. We then analysed the levels of TNF-α mRNA after 6 h of treatment and the levels of TNF-α in supernatants after 24 h. Four independent experiments were performed in duplicates. The expression of TNF-α mRNA was up-regulated by more than three times in monocytes treated with the S100A4 multimer compared with monocytes exposed to the S100A4 dimer ($P < 0.01$) (Fig. 3A). In addition, the synthesis of TNF-α by monocytes was significantly increased in those samples treated with the S100A4 multimer for 24 h, when compared with monocytes stimulated with the S100A4 dimer and to unstimulated monocytes [4474.6 (1282) *vs* 958.4 (706.2) *vs* 250.3 (165) pg/ml; $P < 0.001$] (Fig. 3B).

Discussion

This study shows, for the first time, that the levels of the metastasis-inducing protein, S100A4, are significantly correlated with the disease activity of RA. In addition, we also showed that only the bioactive S100A4 multimer, but not the total amount of S100A4, appears to decline after successful TNF-α blocking therapy with adalimumab.

S100A4 protein has been mostly studied in cancers, particularly with respect to its ability to enhance metastasis [23]. In addition, recent data have linked S100A4 to the pathogenesis of RA. We have demonstrated the up-regulation of S100A4 in the synovium, SF and bloodstream of patients with RA. We have also shown that this protein has a regulatory role within several pathogenic aspects of the disease [19–21]. In contrast with other S100 proteins, S100A4 was not restricted to phagocytes, but also co-localized with fibroblasts, several immune cells and vascular cells [21]. The abundance of S100A4 in synovial tissue and SF from patients with RA was consistent with the finding of high concentrations of the protein in RA, compared with the OA/control plasma samples [21]. Consequently, we have shown, for the first time, a significant association between the S100A4 concentration and the RA disease activity. Our finding is consistent with the data presented by Foell *et al.* [12] and Wulffraat *et al.* [13], who found an association between other S100 proteins

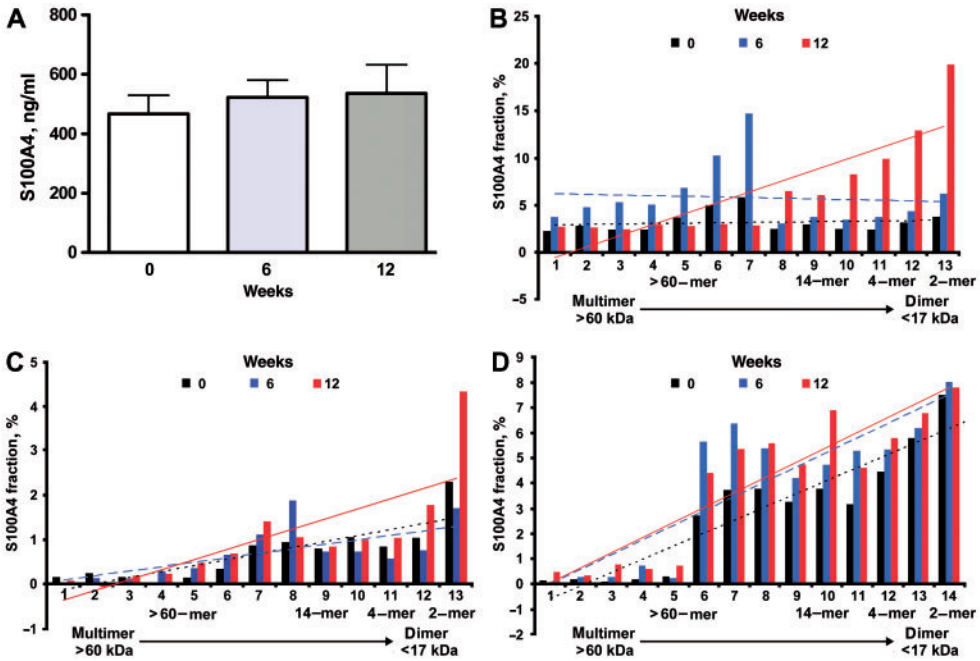

FIG. 2. Modulation of metastasis-inducing protein S100A4 plasma levels in RA patients in response to the TNF-α inhibitor adalimumab. Total plasma levels of S100A4 remained unchanged during the therapy (**A**). An obvious shift occurred in S100A4 from the high to the low molecular weight form, together with a decline in the bioactive S100A4 multimer after 12 weeks of the therapy with adalimumab (**B** and **C**). However, no clear conformational shift of S100A4 from the high to the low (dimeric) molecular weight, but rather a tendency for an increase of some multimeric and oligomeric S100A4 forms, was documented in a patient who experienced an inadequate response to the treatment with adalimumab. The representative profile of the S100A4 conformational modulation is depicted in (**D**). The numbers on the *x*-axis represent 13 fractions of S100A4 with different molecular weights (in kilodaltons) separated by size-exclusion chromatography. Each fraction corresponds to a certain S100A4 conformation, for instance fr 13: 2-mer; fr. 11: 4-mer; fr. 9: 14mer; fr. 5: >60-mer. Trend lines are indicated.

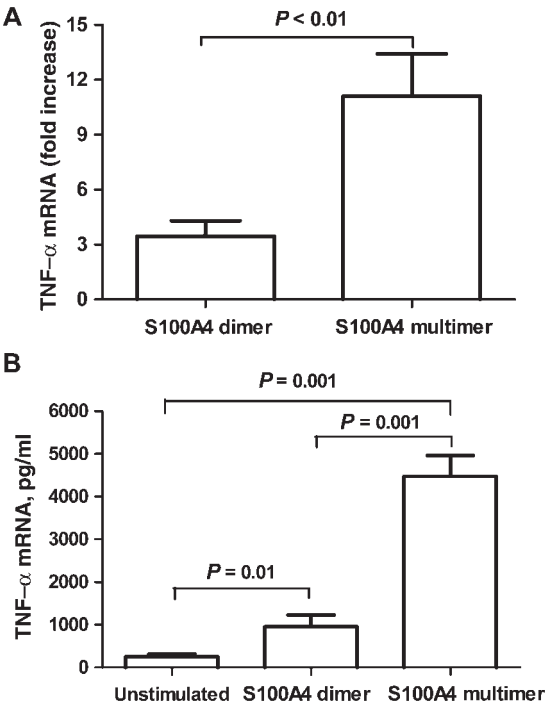

FIG. 3. Increased expression of TNF-α mRNA in human monocytes treated with the S100A4 multimer for 6 h compared with monocytes exposed to the S100A4 dimer (**A**). Monocytes treated with S100A4 multimer for 24 h significantly enhanced TNF-α release into the cell culture medium compared with monocytes stimulated with S100A4 dimer and unstimulated monocytes (**B**). Results of TNF-α gene expression are expressed as fold increases with respect to the expression of TNF-α mRNA in unstimulated monocytes. Data are obtained from four independent experiments performed in duplicates.

(S100A12 and S100A8/9) and disease activity in patients with RA. Furthermore, an association of S100A8/9 with autoantibodies, including RF or ACPA, has been previously documented [29]. Nevertheless, we have found no significant correlation between S100A4 and RF or ACPA antibodies. We have also not found any correlation between S100A4 protein and CRP. Thereby, it can be suggested that S100A4 reflects the disease activity as assessed by all the components of DAS28 rather than the acute phase response or the immune activation only.

It has been previously demonstrated that the concentrations of S100A8/9 and S100A12 decrease significantly both at the local and global (bloodstream) levels in patients with RA, following TNF-α blocking therapy [14, 16, 17]. Therefore, we expected changes in the plasmatic S100A4 levels after TNF-α blocking therapy. Surprisingly, TNF-α blocking with adalimumab did not change plasma levels of the total S100A4 protein. S100A4 can exist as a multimer or as a dimer. Several studies have demonstrated that the dimer form of S100A4 resides intracellularly. In contrast, the multimeric conformations, which are the biologically active forms of S100A4 and which can therefore trigger several cellular responses, reside mostly extracellularly [28, 30]. By analysing the conformation of the S100A4 protein, we observed a conformational shift from the bioactive multimers to the less active, oligomeric and dimeric forms following adalimumab therapy. To confirm the bioactivity of the S100A4 multimer [28, 30], we demonstrated that when compared with the dimer, the S100A4 multimer, considerably up-regulated TNF-α gene expression and protein release by activated monocytes. These results are in agreement with our previous reports showing that only the S100A4 multimer, but not the dimer, was capable of modulating the expression of matrix-degrading enzymes and apoptotic molecules in synovial fibroblasts [19, 21]. Since multimers are considered as biologically active, we hypothesize that the down-regulation of the bioactive S100A4 protein (multimer) could be associated with the improvement in disease activity observed after TNF-α blocking therapy. Moreover, our unpublished *in vitro* data with synovial fibroblasts correspond well with the results of Devoogdt *et al.* [31] in cancer cells, which suggested that TNF-α does not change the expression of S100A4 mRNA. In addition, S100A4 mRNA is up-regulated in monocytes from patients with RA, compared with healthy controls. However, S100A4 mRNA does not appear to be modulated by TNF-α in human monocytes (L Oslejskova, unpublished data). Thus, we suggest that S100A4 is regulated independently of TNF-α, and/or by post-translational modification, depending on the inflammatory activity of the disease.

This study has some limitations. We have not correlated the levels of S100A4 protein with the measures of joint damage. Therefore, we could not postulate the potential predictive value of S100A4 for further development of the disease, as demonstrated recently for calprotectin [29, 32]. It is not yet possible to quantify the S100A4 multimer and associate this form with the disease activity of RA, a relationship that could be extremely informative. Moreover, this study was designed to analyse the association of S100A4 with the disease activity of RA and did not include healthy controls for comparison. However, our previous results have demonstrated a significant increase in plasma S100A4 levels in patients with RA, compared with control individuals [21].

In conclusion, we have demonstrated for the first time that the levels of the metastasis-inducing protein S100A4, similar to alarmins S100A8/9 and S100A12, are significantly correlated with RA disease activity. Furthermore, we have shown that the bioactive S100A4 multimer, but not the total amount of S100A4, declines after successful TNF-α blocking therapy.

Rheumatology key messages

- The levels of the metastasis-inducing protein S100A4 in plasma correlate with RA disease activity.
- Bioactive S100A4 multimer, but not the total amount of S100A4, declines after successful TNF-α blocking therapy.
- S100A4 multimer up-regulates TNF-α gene expression and protein release by activated monocytes *in vitro*.

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Disclosure statement: The authors have declared no conflicts of interest.

References

1 Brennan FM, Foey AD, Feldmann M. The importance of T cell interactions with macrophages in rheumatoid cytokine production. Curr Top Microbiol Immunol 2006;305:177–94.
2 Zouali M. B lymphocytes—chief players and therapeutic targets in autoimmune diseases. Front Biosci 2008;13::4852–61.
3 Huber LC, Distler O, Tarner I, Gay RE, Gay S, Pap T. Synovial fibroblasts: key players in rheumatoid arthritis. Rheumatology 2006;45:669–75.
4 Chu CQ, Field M, Feldmann M, Maini RN. Localization of tumor necrosis factor alpha in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. Arthritis Rheum 1991;34:1125–32.
5 Elliot MJ, Maini RN, Feldmann M *et al.* Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. Arthritis Rheum 1993;36:1681–90.
6 Maini RN, Breedveld FC, Kalden JR *et al.* Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum 1998;41:1552–63.

7 Maini R, St Clair EW, Breedveld F *et al.* Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. Lancet 1999;354:1932–9.
8 Donato R. Intracellular and extracellular roles of S100 proteins. Microsc Res Tech 2003;60:540–51.
9 Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 2007;81:1–5.
10 Foell D, Wittkowski H, Vogl T, Roth J. S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. J Leukoc Biol 2007; 81:28–37.
11 Youssef P, Roth J, Frosch M *et al.* Expression of myeloid related proteins (MRP) 8 and 14 and the MRP8/14 heterodimer in rheumatoid arthritis synovial membrane. J Rheumatol 1999;26:2523–8.
12 Foell D, Kane D, Bresnihan B *et al.* Expression of the pro-inflammatory protein S100A12 (EN-RAGE) in rheumatoid and psoriatic arthritis. Rheumatology 2003;42: 1383–9.
13 Wulffraat NM, Haas PJ, Frosch M *et al.* Myeloid related protein 8 and 14 secretion reflects phagocyte activation and correlates with disease activity in juvenile idiopathic arthritis treated with autologous stem cell transplantation. Ann Rheum Dis 2003;62: 236–41.
14 Foell D, Wittkowski H, Hammerschmidt I *et al.* Monitoring neutrophil activation in juvenile rheumatoid arthritis by S100A12 serum concentrations. Arthritis Rheum 2004;50:1286–95.
15 Schulze zur Wiesch A, Foell D, Frosch M, Vogl T, Sorg C, Roth J. Myeloid related proteins MRP8/MRP14 may predict disease flares in juvenile idiopathic arthritis. Clin Exp Rheumatol 2004;22:368–73.
16 Wittkowski H, Foell D, af Klint E *et al.* Effects of intra-articular corticosteroids and anti-TNF therapy on neutrophil activation in rheumatoid arthritis. Ann Rheum Dis 2007;66:1020–5.
17 De Rycke L, Baeten D, Foell D *et al.* Differential expression and response to anti-TNF alpha treatment of infiltrating versus resident tissue macrophage subsets in autoimmune arthritis. J Pathol 2005;205:17–27.
18 Masuda K, Masuda R, Neidhart M *et al.* Molecular profile of synovial fibroblasts in rheumatoid arthritis depends on the stage of proliferation. Arthritis Res 2002;4:R8.
19 Senolt L, Grigorian M, Lukanidin E *et al.* S100A4 is expressed at site of invasion in rheumatoid arthritis synovium and modulates production of matrix metalloproteinases. Ann Rheum Dis 2006;65:1645–8.
20 Senolt L, Grigorian M, Lukanidin E *et al.* S100A4 (Mts1): is there any relation to the pathogenesis of rheumatoid arthritis? Autoimmun Rev 2006;5:129–31.
21 Klingelhöfer J, Senolt L, Baslund B *et al.* Up-regulation of metastasis-promoting S100A4 (Mts 1) in rheumatoid arthritis: putative involvement in the pathogenesis of RA. Arthritis Rheum 2007;56:779–89.
22 Ebralidze A, Tulchinsky E, Grigorian M *et al.* Isolation and characterization of a gene specifically expressed in different metastatic cells and whose deduced gene product has a high degree of homology to a Ca2+-binding protein family. Genes Dev 1989;3: 1086–93.
23 Helfman DM, Kim EJ, Lukanidin E, Grigorian M. The metastasis associated protein S100A4: role in tumour progression and metastasis. Br J Cancer 2005;92: 1955–8.
24 Yammani RR, Carlson CS, Bresnick AR, Loeser RF. Increase in production of matrix metalloproteinase 13 by human articular chondrocytes due to stimulation with S100A4: role of the receptor for advanced glycation end products. Arthritis Rheum 2006;54:2901–11.
25 Oslejskova L, Grigorian M, Gay S, Neidhart M, Senolt L. The metastasis associated protein S100A4: a potential novel link to inflammation and consequent aggressive behavior of rheumatoid arthritis synovial fibroblasts. Ann Rheum Dis 2008;67: 1499–504.
26 Arnett FC, Edworthy SM, Bloch DA *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
27 Prevoo ML, van't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Arthritis Rheum 1995;38:44–8.
28 Novitskaya V, Grigorian M, Kriajevska M *et al.* Oligomeric forms of the metastasis-related Mts1 (S100A4) protein stimulate neuronal differentiation in cultures of rat hippocampal neurons. J Biol Chem 2000;275:41278–86.
29 Hammer HB, Odegard S, Syversen SW *et al.* Calprotectin (a major S100 leukocyte protein) predicts 10-year radiographic progression in patients with rheumatoid arthritis. Ann Rheum Dis, 2008 18 December [Epub ahead of print].
30 Ornås M, Tulchinsky E, Lukanidin E, Ambartsumian N. Extracellular S100A4 (mts1) stimulates invasive growth of mouse endothelial cells and modulates MMP-13 matrix metalloproteinase activity. Oncogene 2004;23:5487–95.
31 Devoogdt N, Revets H, Kindt A, Liu YQ, De Baetselier P, Ghassabeh GH. The tumor-promoting effect of TNF-alpha involves the induction of secretory leukocyte protease inhibitor. J Immunol 2006;177:8046–52.
32 Hammer HB, Odegard S, Fagerhol MK *et al.* Calprotectin (a major leucocyte protein) is strongly and independently correlated with joint inflammation and damage in rheumatoid arthritis. Ann Rheum Dis 2007;66:1093–7.