Decreased plasma levels of soluble CD18 link leukocyte infiltration with disease activity in spondyloarthritis

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Abstract

Introduction: Spondyloarthritis (SpA) comprises a group of diseases often associated with HLA-B27 and characterized by inflammation of the entheses and joints of the axial skeleton. The inflammatory process in SpA is presumably driven by innate immune cells but is still poorly understood. Thus, new tools for monitoring and treating inflammation are needed. The family of CD18 integrins is pivotal in guiding leukocytes to sites of inflammation, and CD18 hypomorphic mice develop a disease resembling SpA. Previously, we demonstrated that altered soluble CD18 (sCD18) complexes in the blood and synovial fluid of patients with arthritis have anti-inflammatory functions. Here, we study the mechanisms for these alterations and their association with SpA disease activity.

Methods: Plasma levels of sCD18 in a study population with 84 patients with SpA and matched healthy controls were analyzed with a time-resolved immunoflourometric assay (TRIFMA). Binding of sCD18 to endothelial cells and fibroblast-like synoviocytes (FLSs) was studied with confocal microscopy. Shedding of CD18 from peripheral blood mononuclear cells (PBMCs) was studied with flow cytometry and TRIFMA.

Results: Plasma levels of sCD18 were decreased in patients with SpA compared with healthy volunteers (P<0.001), and the lowest levels were in the HLA-B27-positive subgroup (P<0.05). In a multiple regression model, the sCD18 levels exhibited an inverse correlation with the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (P<0.05), the level of morning stiffness (P<0.05), the Bath Ankylosing Spondilitis Metrology Index (P<0.05), the physician global assessment score (P<0.01), and the sacroiliac magnetic resonance imaging activity score (P<0.05). The mechanisms for these changes could be simulated in vitro. First, sCD18 in plasma adhered to inflammation-induced intercellular adhesion molecule 1 (ICAM-1) on endothelial cells and FLS, indicating increased consumption. Second, CD18 shedding from SpA PBMCs correlated inversely with the BASDAI (P<0.05), suggesting insufficient generation. CD18 was shed primarily from intermediate CD14++ CD16+ monocytes, supporting the view that alterations in innate immunity can regulate the inflammatory processes in SpA.

Conclusions: Taken together, the failure of patients with SpA to maintain adequate sCD18 levels may reflect insufficient CD18 shedding from monocytes to counterbalance the capture of sCD18 complexes to inflammation-induced ICAM-1. This could increase the availability of ICAM-1 molecules on the endothelium and in the synovium, facilitating leukocyte migration to the entheses and joints and aggregating disease activity.

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Introduction
Spondyloarthritis (SpA) comprises a group of common inflammatory musculoskeletal diseases, including ankylosing spondylitis, psoriatic arthritis, arthritis associated with inflammatory bowel disease, reactive arthritis, and undifferentiated SpA. Patients with SpA share the characteristics of a high frequency of the HLA-B27 allele in the MHC I loci and disease affecting the entheses and joints of the axial skeleton [1]. In SpA, disease disability is the sum of the inflammatory burden and chronic changes. Thus, distinguishing between inflammatory activity and chronic problems is critical for choosing a proper clinical intervention [2,3].

The inflammatory process in SpA involves innate immune cells such as macrophages, monocytes, and fibroblasts [4]. Monocytes are present in the blood and can be divided into the three subsets: CD14++ CD16− classic monocytes, CD14++ CD16+ intermediate monocytes, and CD14+ CD16++ non-classic monocytes [5]. The intermediate monocyte subset increases during an infection [6] and is elevated in RA and SpA [7-9]. Intermediate monocytes have been described to have both pro-inflammatory and anti-inflammatory activities [5], and the role of intermediate monocytes in arthritis is still elusive. Disease activity in SpA is associated with migration of leukocytes to inflamed joints and entheses [1]. However, the complexity of the inflammatory process in SpA is still poorly understood.

The β2 (CD18) family of integrins includes CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1 or complement receptor [CR] 3), CD11c/CD18 (CR4, p150,95), and CD11d/CD18 (LFA-1), CD11b/CD18 (Mac-1 or complement receptor α, p112) complexes of CD11/CD18. These complexes bind ICAM-1 as well as activation of integrins for ligand binding [10,11]. In arthritis, the increased expression of ICAM-1 results in the migration of leukocytes to the joints [12]. Animal studies support a central role of CD18 in the development of SpA. The mouse PL/J strain carries the CD18 hypomorphic mutation, which reduces the expression of CD18 to 2% to 16% of wild-type levels [13]. These mice develop a skin disease that closely resembles human psoriasis together with a condition very similar to psoriatic arthritis dominated by enthesitis [14], and the inflammatory response critically involves molecules on the endothelium and in the synovium could facilitate increased leukocyte infiltration in the entheses and joints and increased disease activity. Hereby, our findings link regulation of leukocyte infiltration by innate immune cells with inflammation and disease activity in SpA.

Materials and methods
Study populations
At the time of inclusion, the study population comprised 84 patients with axial SpA according to the European Spondyloarthropathy Study Group (ESSG) criteria [28,29]. At a follow-up 4 years later, 43 of these patients were examined again (Table 1). A total of 47 patients met the modified New York criteria for ankylosing spondylitis at the time of inclusion [28,29], whereas 37 patients had non-radiographic SpA. The study population was clinically well characterized, and self-assessment scores and clinical scores comprised physician global assessment visual analogue scale (VAS), patient pain VAS, patient global VAS, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Metrology Index (BASMI), thoracic chest expansion and disease duration at both baseline and follow-up. The test results included C-reactive protein (CRP), HLA-B27 status, radiography, and magnetic resonance imaging (MRI). MRI of the sacroiliac...
joints (SJs) and the entire spine at both baseline and follow-up and radiography of the SIJ at baseline were included in this study (Table 1). A specialist radiologist who was experienced in SpA but who had no knowledge of the clinical findings analyzed all MRI and radiographic tests. The MRI changes were graded by using methods previously described [30,31]. In summary, the MRI SJJ and spinal activity scores were made in accordance with the Danish methods primarily differing from the Spondyloarthritis Research Consortium of Canada (SPARCC) methods by using fully three-dimensional assessment of the SIJ and the spine [32,33]. The intra- and inter-observer agreements of the methods have been demonstrated to be acceptable [30,31].

One SpA patient with peripheral arthritis was included for growing fibroblast-like synoviocytes (FLSs) from synovial fluid mononuclear cells (SFMCs). The patient contacted the out-patient clinic because of a knee joint

### Table 1 Patient characteristics

| Characteristics                          | At time of inclusion (n = 84) | At follow-up (n = 43) | HCs (n = 28) |
|-----------------------------------------|-----------------------------|----------------------|-------------|
| Age in years (mean)                     | 37 (35-39)                  | 41 (38-43)           | 42.9        |
| Gender, percentage female               | 58                          | 59                   | 57          |
| Diagnosis, percentage of patients       |                             |                      |             |
| Ankylosing spondylitis                  | 19                          | 19                   | -           |
| Psoriatic arthritis                     | 11                          | 12                   | -           |
| Enteropathic arthritis                  | 4                           | 5                    | -           |
| Reactive arthritis                      | 15                          | 17                   | -           |
| Undifferentiated SpA                    | 51                          | 47                   | -           |
| HLA-B27, percentage positive            | 60                          | 55                   | -           |
| Disease duration in years (mean)*       | 8.0 (7.0-9.0)               | 12.6 (9.8-15)        | -           |
| Treatment, percentage of patients       |                             |                      |             |
| No                                      | 67                          | -                    | -           |
| MTX                                     | 8                           | -                    | -           |
| Salazopyrin                             | 11                          | -                    | -           |
| Anti-TNFα                                | 7                           | 12                   | -           |
| Self-assessment scores                  |                             |                      |             |
| BASDAI, 0-100 (mean)                    | 32 (26-37)                  | 35 (28-42)           | -           |
| BASFI, 0-100 (mean)                     | 20 (16-25)                  | 22 (16-28)           | -           |
| Patient pain, 0-100 (mean)              | 32 (26-38)                  | 33 (26-41)           | -           |
| Patient global, 0-100 (mean)            | 32 (26-38)                  | 31 (23-38)           | -           |
| Level of morning stiffness, 0-100 (mean)| 36 (29-43)                  | 36 (27-44)           | -           |
| Duration of morning stiffness, 0-100 (mean)| 33 (26-41)      | 29 (20-38)           | -           |
| Clinical scores                         |                             |                      |             |
| BASMI, 0-100 (median)                   | 0 (0-0)                     | 0 (0-10)             | -           |
| Physician global, 0-100 (mean)          | 16 (13-20)                  | 20 (16-25)           | -           |
| Thoracic chest expansion in cm (mean)   | 4.1 (3.9-4.4)               | 4.9 (4.5-5.3)        | -           |
| Test results                             |                             |                      |             |
| CRP in mg/L (median)                    | 2.1 (1.3-3.9)               | 1.0 (0.5-2.5)        | -           |
| SJ MRI activity, 0-40 (mean)            | 7.5 (5.9-9.0)               | 4.3 (2.9-5.7)        | -           |
| Spine MRI activity, 0-81 (median)       | 1 (0-4)                     | 1 (0-2)              | -           |
| SJ MRI chronicity, 0-48 (mean)          | 15 (12-18)                  | 17 (13-22)           | -           |
| Spine MRI chronicity, 0-207 (median)    | 0 (0-4)                     | 0 (0-5)              | -           |

Numbers in parentheses are 95% confidence interval for measures with a Gaussian distribution and 25% to 75% percentiles for measures with a non-Gaussian distribution. *Disease duration was defined as duration since start of symptoms. -, not available; Anti-TNF-α, anti-tumor necrosis factor-alpha; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; CRP, C-reactive protein; HC, healthy control; MRI, magnetic resonance imaging; MTX, methotrexate; SIJ, sacroiliac joint; SpA, spondyloarthritis.
effusion. No disease activity or prognosis scores or test results were recorded.

Plasma from age- and gender-matched healthy controls (HCs) from either the Blood Donor Bank at Aarhus University Hospital or from patients undergoing orthopedic surgery at the Department of Orthopaedics at Aarhus University Hospital (n = 28) was included for measuring sCD18 plasma concentration (Table 1). Peripheral blood mononuclear cells (PBMCs) from HCs were included for in vitro studies of spontaneous shedding of CD18 from PBMCs, T cells, natural killer (NK) cells, and monocytes.

All plasma samples were collected in heparinized tubes and kept at −80°C until use. PBMCs and SFMCs were isolated by conventional Ficoll-Paque (GE Healthcare, Little Chalfont, Buckinghamshire, UK) density-gradient centrifugation and cryopreserved at −135°C until time of analysis. HC PBMCs were isolated from buffy coats for the in vitro studies of spontaneous shedding of CD18.

**Quantification of sCD18 and MMP-9 by time-resolved immunofluorometric assays**

The IgG1 monoclonal antibodies (mAbs) against CD18 were produced by GenScript from the hybridoma cell lines CRL-2839 (KIM185) and CRL-2838 (KIM127) followed by protein A/G purification. The mouse IgG1 isotype (cat. no. M7894; Sigma-Aldrich, St. Louis, MO, USA) was purified from an ascites suspension with protein A/G purification. Biotinylated forms of the antibodies were made by use of Biotin N-hydroxysuccinimide ester (cat. no. 14405; Sigma-Aldrich) [18].

Detection of sCD18 in plasma samples and supernatants from in vitro cell cultures was carried out by time-resolved immunofluorometric assays (TRIFMAs) by using a sandwich technique as previously described [18]. Briefly, microtiter wells (FluoroNunc Maxisorp, 437958; Nunc, Roskilde, Denmark) were coated with antibody to CD18 (KIM185) or mouse IgG1. The wells were washed in Tris-buffered saline (TBS)/Tween and blocked by incubation with 200 μL TBS with 1 mg/mL human serum albumin for 1 hour at room temperature (RT). After washes, samples of 100 μL heparinized plasma diluted 1:10 and 1:5 or supernatants diluted 1:2 in TBS/Tween with 1 mM CaCl₂, 1 mM MgCl₂, and 100 μg/mL aggregated human Ig were added to the wells, and the plates were incubated overnight at 4°C. After incubation of the diluted samples, the wells were washed and subsequently incubated with 100 μL biotinylated antibody to CD18 (KIM127). After washing of the wells, Eu³⁺-conjugated streptavidin was applied and the signals were read by time-resolved fluorometry. Signals from patient samples were compared against a standard curve made from titrations of the HC plasma also used for sCD18 measurements. As a control, plates coated with murine IgG1 isotype were prepared and the signals from these wells were subtracted from the signals recorded in wells with the mAb to MMP-9.

**Confocal microscopy of sCD18 binding to ICAM-1 expressed on EA.hy926 cells and SpA FLSs**

The EA.hy926 endothelial cell line was cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (vol/vol) fetal calf serum (FCS), penicillin, streptomycin, and glutamine. FLSs were grown from SFMCs as done previously [34]. Briefly, SFMCs were isolated and cryopreserved as described above. The cells were thawed and cultured in supplemented DMEM at a concentration of 2 × 10⁶ cells/mL at 37°C in a humidified incubator with 5% CO₂ (vol/vol) replacing the medium every 3 to 4 days. When the cell layer was 70% confluent, the FLSs were passaged by trypsin/ethylenediaminetetra-acetic acid (EDTA) treatment and used for analyses at passage 5. Sterile glass slides were placed in 24-well cell culture plates and aseptically treated with 300 μL of 0.1% (wt/vol) polylysine dissolved in water (cat. no. P8920; Sigma-Aldrich) for 5 minutes. After thorough rinsing with sterile water, the glass slides were allowed to dry for 2 hours. Either EA.hy926 cells or FLSs were then seeded at a concentration of 2.5 × 10⁴ cells/mL in supplemented DMEM and incubated for 24 hours at 37°C. The cells were either stimulated with TNFα (cat. no. 300-01A; PeproTech, Rocky Hill, NJ, USA) at a concentration of 10 ng/mL or incubated with medium alone for 24 hours at 37°C. After aspiration of the culture medium, cells were incubated with 50% (vol/vol) heat-inactivated HC serum with a known concentration of sCD11/CD18 (542 mU sCD18 per mL) in 150 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, 1 mM MnCl₂, 10 mM HEPES, pH 7.4 (Binding Buffer). As another source of sCD11/CD18, additional incubations were made with 50% (vol/vol) SFMC culture supernatant with sCD11/CD18 (approximately 25 mU/mL) in Binding Buffer or Binding Buffer alone for 16 hours at 37°C. Wells were washed twice, and cells were fixed with 300 μL 3.7% (vol/vol) paraformaldehyde for 10 minutes at RT. Unspecific binding was blocked with both 10 μg/mL murine IgG and 10 μg/mL bovine IgG in
200 μL Binding Buffer for 30 minutes at RT. Cells were stained with either biotinylated mouse IgG1 anti-CD18 (KIM127) or biotinylated mouse IgG1 isotype in combination with streptavidin Alexa flour 546 (cat. no. F7143; Dako, Glostrup, Denmark). Wells were washed twice, and glass slides were placed in 2 μL of anti-fade mounting medium with 1/1,000 4',6-diamidino-2-phenylindole (DAPI) (cat. no. D9542; Sigma-Aldrich) and allowed to dry overnight. All micrographs were collected by using a Zeiss LSM-710 confocal microscope and Pixelmator (Pixelmator Team Ltd., Vilnius, Lithuania) for editing.

Flow cytometric analyses of PBMCs
Cells were transferred to FACS tubes (Nunc) and blocked for unspecific binding in phosphate-buffered saline with 0.5% (wt/vol) bovine serum albumin (cat. no. 12659; Calbiochem, now part of EMD Biosciences, Inc., San Diego, CA, USA), 0.09% (wt/vol) NaN3 together with 10 μg/mL murine IgG and 10 μg/mL bovine IgG (cat. nos. 015-000-003 and 001-000-003; Jackson ImmunoResearch, West Grove, PA, USA). Cells were stained with either biotinylated mouse IgG1 mAb to CD18 (KIM185) or biotinylated mouse IgG1 isotypic control in combination with streptavidin-FITC (Dako). According to the manufacturer’s instructions, mouse IgG1 anti-CD163 PE antibody (clone MAC2-158, cat. no. CD163-158P; Trillium Diagnostics), mouse IgG2a anti-CD14 ECD antibody (clone RMO52, cat. no. PN IM2707U; Beckman Coulter), mouse IgG1 anti-CD16 APC antibody (clone eBioCB16, cat. no. 17-0168; eBioscience, San Diego, CA, USA), mouse IgG1 anti-CD3 ECD antibody (clone UCHT1, cat. no. A07748; Immunotech, Beckman Coulter), and mouse IgG2b anti-CD56 antibody (clone c5.9, cat. no. R7251; Dako) were used for subanalysis of the PBMCs, and the Live/Dead staining (cat. no. L10119; Invitrogen) was used for viability. All samples were analyzed within 24 hours by using an FC500 with CXP software (Beckman Coulter) and FlowJo software version 9.6 (Tree Star Inc., Ashland, OR, USA).

In vitro culture experiments with PBMCs
For in vitro culture experiments with PBMCs, the cells were thawed and cultured in RPMI medium supplemented with 10% (vol/vol) FCS, penicillin, streptomycin, and glutamine. To study differences in spontaneous CD18 shedding between SpA and HC unfractionated PBMCs, the cells were incubated at a concentration of 1 × 10^6 cells/mL. To study differences in spontaneous CD18 shedding from PBMC subsets, T cells (cat. no. 130-091-156; Miltenyi Biotec, Bergisch Gladbach, Germany), NK cells (cat. no. 130-092-657; Miltenyi Biotec), and monocytes (cat. no. 130-091-153; Miltenyi Biotec) were isolated by negative selection in accordance with the instructions of the manufacturer and incubated at a concentration of 1 × 10^6 cells/mL. In all PBMC experiments, cells were cultured for 48 hours at 37°C in a humidified incubator 5% (vol/vol) CO₂ without changing of medium. After incubation, supernatants were stored frozen at −80°C for later sCD18 analysis with TRIFMA.

Statistical analyses
Gaussian distributed measures were presented by the mean value, whereas measures with a non-Gaussian distribution were presented by the median value as indicated. Prior to analyses for statistical significance, the plasma sCD18 levels were log-transformed. Comparisons of the plasma sCD18 levels between groups of unpaired and paired samples were made by using the Student t test in the unpaired and paired modes, respectively. Correlation analyses between disease activity parameters and the plasma sCD18 levels were performed with the Pearson correlation for measures with a Gaussian distribution and with the Spearman correlation for measures with a non-Gaussian distribution. Multiple regression models were made with the plasma sCD18 levels and disease activity parameters correcting for age, disease duration, HLA-B27 status, and treatment at time of inclusion. The same analyses were made after additional correction for CRP. All cell culture experiments were analyzed with non-parametric statistics. The Mann-Whitney U test was used for unpaired comparisons, the Wilcoxon signed rank test was used for paired data, and the Spearman correlation was used for association studies. A two-tailed P value below 0.05 was considered significant. Calculations and graphs were made with Stata version 11.1 (StataCorp LP, College Station, TX, USA) and GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA).

Approval of studies using human samples
All SpA samples were collected in the out-patient clinic in Aarhus University Hospital or in the Aarhus Rheumatology Clinic private practice. All samples were obtained after informed written consent according to the Declaration of Helsinki and approved by the Research Ethics Committees of Central Jutland (project numbers 20050046 and 20058432) and the Danish Data Protection Agency.

Results
The plasma level of sCD18 was lower in SpA patients compared with healthy controls
We measured the concentration of sCD18 in plasma samples from patients with SpA and HCs by using TRIFMA. The levels of sCD18 in plasma from patients with SpA at both time of inclusion and 4-year follow-up were significantly lower than in plasma from HCs (Figure 1). Furthermore, the levels of sCD18 showed a clear overlap between the three groups. There was no significant difference
between the plasma sCD18 levels in patients with SpA at time of inclusion compared with the second time-point 4 years later ($P = 0.10$) (Figure 1). No associations were observed between gender or age and plasma sCD18 levels for either patients with SpA or HCs.

The plasma level of sCD18 was negatively associated with disease activity and decreased in HLA-B27-positive SpA. We correlated the sCD18 concentration with several disease activity parameters to analyze for an association between decrease in sCD18 plasma level and disease activity. These included scores calculated from information provided by the patients (BASDAI, BASFI, patient pain VAS, patient global assessment score, and morning stiffness), scores resulting from clinical examination (BASMI, physician global assessment score, and thoracic chest expansion), and objective measures (CRP, sacroiliac joint [SII] MRI activity score, and spine MRI activity score).

Significant negative correlations were observed between the plasma sCD18 levels and BASMI ($r = -0.25$, $P < 0.05$), physician global assessment score ($r = -0.34$, $P < 0.01$), CRP ($r = -0.34$, $P < 0.01$), and SII MRI activity score ($r = -0.25$, $P < 0.05$). No correlations were observed between sCD18 and MRI chronicity scores.

The association of sCD18 plasma level and disease activity was further investigated by using subgroup and multivariate analyses. Overall, the levels of sCD18 in plasma from HLA-B27-positive SpA patients were reduced compared with HLA-B27-negative SpA patients (Figure 2A). No differences in plasma sCD18 levels were observed when comparing the SpA subtypes (ankylosing spondylitis, psoriatic arthritis, enteropathic arthritis, reactive arthritis, and undifferentiated SpA), when comparing patients fulfilling or not fulfilling the modified New York criteria for ankylosing spondylitis, or when comparing patients treated or not treated with a TNFα inhibitor. Because HLA-B27 positivity adds to the strength of the SpA diagnosis and because we found an association between the sCD18 plasma concentration and HLA-B27 positivity, the HLA-B27-positive SpA patients were analyzed separately. In this subgroup, significant negative correlations were present between the plasma sCD18 levels and BASDAI ($r = -0.39$), BASMI ($r = -0.38$), level of morning stiffness ($r = -0.43$), duration of morning stiffness ($r = -0.33$), physician global assessment score ($r = -0.62$), CRP ($r = -0.38$), and SII MRI activity score ($r = -0.35$) while thoracic chest expansion showed a positive association to the plasma sCD18 level ($r = 0.37$) (Figure 2B and C).

The association between plasma sCD18 levels and markers of disease activity was also tested in regression models with correction for age, disease duration, HLA-B27 status, and treatment at time of inclusion. In these models, significant negative correlations were observed between the plasma sCD18 levels and BASDAI, level of morning stiffness, physician global assessment score, BASMI, and SII MRI activity score (Tables 2 and 3). Furthermore, we analyzed these associations after correcting for CRP to evaluate whether sCD18 adds to the information already achieved by the CRP. The associations remained significant for BASDAI, level of morning stiffness, BASMI, and physician global assessment score (Additional file 1: Table S1 and Additional file 2: Table S2). The same tendencies were seen between plasma sCD18 and markers of disease activity at 4-year follow-up, but these were not significant (data not shown).

The value of sCD18 plasma concentration as a prognostic marker in SpA was tested by correlating the plasma sCD18 concentration at baseline with disease activity scores at the 4-year follow-up (BASDAI, BASFI, patient pain VAS, patient global assessment score, morning stiffness, BASMI, physician global assessment score, thoracic chest expansion, CRP, SII MRI activity score, and spine
MRI activity score) and changes in these parameters from the inclusion to the 4-year follow-up. When the entire group of SpA patients was examined, there were no significant associations. When only the HLA-B27-positive SpA patients were examined, there was a significant negative correlation between the plasma sCD18 levels at time of inclusion and disease activity at 4-year follow-up measured by BASDAI ($r = -0.42$, $P < 0.05$).

sCD11/CD18 complexes were captured by ICAM-1 on EA.hy926 cells and SpA FLSs

To provide a rationale for the negative relation between plasma sCD18 levels and disease activity, the ability of sCD18 to bind inflammation-induced ICAM-1 was studied as outlined in the illustration (Figure 3A). First, cells were stimulated with TNFα to increase the ICAM-1 expression in the membrane. Second, incubations were made with sCD18. Third, the cells were stained for captured sCD18. A clear signal for sCD18 capture was obtained when staining the endothelial cell line EA.hy926 after stimulation of the cells with TNFα and incubation with normal human serum (NHS) as the sCD18 source (Figure 3B). In the absence of TNFα stimulation, no staining was observed (Figure 3C). As further controls, the cells were incubated with medium without sCD18 or stained with

Table 2 Associations at time of inclusion between plasma soluble CD18 levels in all spondyloarthritis patients and self-assessment scores after correction for age, disease duration, HLA-B27 status, and treatment

| sCD18 | BASDAI | BASFI | Patient pain | Patient global | Morning stiffness, level | Morning stiffness, duration |
|-------|--------|-------|--------------|----------------|--------------------------|-----------------------------|
| r     | -0.29  | -0.083| -0.14        | -0.11          | -0.27                    | -0.15                       |
| P     | 0.026  | 0.54  | 0.30         | 0.42           | 0.042                    | 0.26                        |

Bold numbers indicate significant correlations. *Partial correlation coefficients. BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; sCD18, soluble CD18.
Table 3 Associations at time of inclusion between plasma soluble CD18 levels in all spondyloarthritis patients and clinical scores and test results after correction for age, disease duration, HLA-B27 status, and treatment.

| sCD18  | Physician global | Thoracic chest expansion | CRP     | SIJ activity | Spine activity |
|--------|------------------|--------------------------|---------|--------------|----------------|
| r      | −0.030           | −0.42                    | 0.12    | −0.23        | −0.27          |
| P      | 0.015            | 0.001                    | 0.34    | 0.069        | 0.032          |

Bold numbers indicate significant associations. *Partial correlation coefficients. BASMI, Bath Ankylosing Spondilitis Metrology Index; CRP, C-reactive protein; sCD18, soluble CD18; SIJ, sacroiliac joint.

Figure 3 Confocal microscopy analysis of the ability of sCD11/CD18 complexes to bind intercellular adhesion molecule 1 (ICAM-1) expressed on the human umbilical vein cell line EA.hy926. (A) Illustration of the cellular incubations. In step 1, adherent cells were stimulated with 10 ng/mL tumor necrosis factor-alpha (TNFα), which increased the ICAM-1 expression. In step 2, a source of CD11/CD18 was added (that is, either normal human serum (NHS) or supernatant from synovial fluid mononuclear cell culture). In step 3, biotinylated antibody recognizing ligand-binding activated CD11/CD18 (KIM127) was added followed by addition of fluorochrom-labelled streptavidin for detection using confocal microscopy. Binding of sCD18 to ICAM-1 expressed on EA.hy926 cells incubated with (B) or without (C) TNFα. Red staining indicates the binding of sCD18, further indicated with white arrows. The positions of cell nuclei were located by 4′,6-diamidino-2-phenylindole (DAPI) staining, indicated in blue. The staining was distinctly localized to small foci on the cell membrane on 10% to 15% of the cells. Expression of ICAM-1 on EA.hy926 cells incubated with (D) or without (E) TNFα. ICAM-1 is indicated with a green staining, and cell nuclei are indicated with a blue staining.
CD18 shedding from PBMCs was affected by donor disease activity

To further analyze the negative relation between plasma sCD18 levels and disease activity, the CD18 shedding was studied in cultures with PBMCs derived from patients with SpA or HCs. In a simple comparison, the spontaneous shedding of CD18 from PBMCs derived from patients with SpA was increased compared with cultures of PBMCs from HCs ($P < 0.01$). In subgroup analysis, the spontaneous shedding of CD18 from PBMCs derived from HLA-B27-negative SpA patients was increased compared with cultures of PBMCs from HCs (Figure 4A), but there were no differences between HLA-B27-positive SpA patients and HCs ($P = 0.69$) or between HLA-B27-negative SpA patients and HLA-B27-positive SpA patients ($P = 0.09$).

To clarify whether shedding of CD18 relates to disease activity in SpA, we correlated shedding of CD18 from SpA patient-derived PBMCs with disease activity scores. The sCD18 concentrations in SpA PBMC supernatants correlated negatively with the BASDAI score (Figure 4B; $r = -0.71$, $P < 0.05$). A similar trend of negative correlation with the sCD18 plasma concentration was found for the other disease scores recorded in this study (data not shown). Since CD18 is a substrate for MMP-9, we measured the concentration of MMP-9 in the same PBMC supernatants. In this case, the analysis presented a non-significant tendency of increasing sCD18 levels with increasing MMP-9 in supernatant from SpA PBMCs (Figure 4C; $r = 0.58$, $P = 0.08$). Taken together, these data suggest that insufficient generation of sCD18 in SpA could be part of an increased disease activity.

CD18 was shed primarily from monocytes

To more clearly identify the cellular sources of sCD18, the cell membrane expression and in vitro shedding were studied with HC- and SpA-derived PBMCs. For both HC- and SpA-derived PBMCs, the cell surface expression of CD18 was higher on monocytes compared with both T cells and NK cells as judged from the median fluorescence intensity (MFI). NK cells also showed a higher cell surface expression of CD18 compared with T cells (Figure 5A and B). The MFIs of the isotype control IgG1 staining were $0.406$, $0.414$, and $2.46$ for T cells, NK cells, and monocytes, respectively. To study the capacity for shedding by these subsets, the cells were separated by application of antibodies to the canonical markers CD14, CD3, or CD56 of monocytes, T cells, and NK cells, respectively. The median percentages of CD14$^+$ cells were $84.3$% (interquartile range $82.8$%-89.8%) in the isolated monocytes, $97.5$% (interquartile range 96.6%-97.7%) of CD3$^+$ cells in the isolated T cells, and $85.9$% (interquartile range 84.1%-90.7%) of CD56$^+$ cells in the isolated NK cells. As expected from the high CD18 cell surface expression, supernatants from monocyte cultures contained a higher sCD18 concentration than supernatants from both T cells and NK cells (Figure 5C).

Since monocytes had the highest CD18 cell membrane expression and showed the highest degree of CD18 shedding, we studied the monocyte subpopulations in further detail. Based on past investigations, the CD14$^+$ cells were stratified into subsets of classic, intermediate, and non-classic monocytes by additional staining for cell membrane expression of CD16. The expression of CD18 was greater on CD14$^{++}$ CD16$^-$ (intermediate) monocytes compared with both CD14$^+$ CD16$^+$ (non-classic) monocytes and CD14$^{++}$ CD16$^-$ (classic) monocytes and was greater on non-classic monocytes compared with classic monocytes (Figure 6A and B). The CD14$^+$ cells were also additionally stained for cell membrane expression of CD163. The expression of CD18 was stronger on CD163$^+$ than CD163$^-$ monocytes ($P < 0.001$). Confirming the finding from previous studies of arthritis [7-9], there were a non-significant, greater proportion of intermediate monocytes among all monocytes in HLA-B27-negative SpA patients compared with HCs (Figure 6C). A positive correlation was observed between the proportion of non-classic (Figure 7A) and intermediate (Figure 7B) monocytes among all CD14$^+$ monocytes and the concentration of sCD18 in the PBMC...
Figure 4 Spontaneous shedding of CD18 from peripheral blood mononuclear cells (PBMCs) cultured in vitro. (A) Levels of spontaneous shedding of CD18 from spondyloarthritis (SpA) and healthy control (HC) PBMCs. The median values were 22.0 mU/mL (interquartile range 14.0-51.8 mU/mL) for HLA-B27-positive SpA patients, 51.7 mU/mL (interquartile range 21.5-57.0 mU/mL) for HLA-B27-negative SpA patients, and 11.2 mU/mL (interquartile range 8.59-12.3 mU/mL) for HCs. PBMCs from 5 HLA-B27-positive SpA patients, 5 HLA-B27-negative patients, and 5 HCs were used. Bars indicate median and interquartile range. (B) Correlation between PBMC donor BASDAI score and the spontaneously shed CD18 in the PBMC culture supernatant. (C) CD18 spontaneous shedding capacity and correlation with matrix metalloproteinase-9 (MMP-9) production. PBMCs from 10 patients with SpA were used. Hatched horizontal lines connect identical measurements of sCD18. Solid black lines represent the best fit in linear regression. *P <0.05.

Figure 5 Cellular-expressed and shed CD18 attributed to peripheral blood mononuclear cell (PBMC) source. (A) The cell membrane expression of CD18 on T cells, natural killer (NK) cells, and monocytes using PBMCs from a representative healthy control (HC) with the gating strategy indicated. (B) The cell membrane expression of CD18 on T cells, NK cells, and monocytes using PBMCs from 10 HCs and 2 patients with spondyloarthritis (SpA). Levels of CD18 expression were measured by the median fluorescence intensity (MFI). Bars indicate median and interquartile range. (C) The concentration of sCD18 in supernatants from cultured T cells, NK cells, and monocytes derived from PBMCs from 5 HCs. Bars indicate median and interquartile range. ***P <0.001.
culture supernatants, whereas a negative correlation was found for the proportion of classic monocytes (Figure 7C).

**Discussion**

The inflammatory process in SpA is still poorly elucidated, resulting in difficulties in monitoring disease activity and choosing treatment strategy. Several investigations in experimental animal models already indicate a central role of CD18 integrins in SpA-like disease [14-16,37]. We previously demonstrated alterations in the sCD18 complexes in the blood and synovial fluid of patients with arthritis and the ability of sCD18 to inhibit leukocyte binding to ICAM-1. This study proposes sCD18 as a novel player in SpA disease pathogenesis. The sCD18 plasma level was decreased in patients with SpA compared with HCs, and levels correlated inversely with SpA disease activity scores. The failure of patients with SpA to maintain normal sCD18 levels seems to reflect insufficient CD18 shedding from monocytes to counterbalance the capture of sCD18 complexes to inflammation-induced ICAM-1. In this way, our findings on sCD18 link increased leukocyte infiltration potential with disease activity in SpA.

As noted by Feldmann and colleagues [38], inflammation is regulated by the balance between pro- and anti-inflammatory mechanisms. In this sense, the inflammatory response may originate as a consequence of over-activity of pro-inflammatory mechanisms or mechanistic failures in anti-inflammatory responses. Previously, it was rather speculative to assign the production of sCD18 to either one of the two modes of regulating the inflammatory response. Yet the ability of sCD11/CD18 complexes to compete with cellular-expressed CD11a/CD18 in ICAM-1 binding [18] indicates that the soluble complexes may aid in resolving cellular adhesion and hence the formation of inflammatory foci. We now found at least two indications to support such anti-inflammatory functions of sCD11/CD18.

First, we demonstrated the ability of the sCD11/CD18 complexes to bind ICAM-1 in the cell membrane of a human umbilical vein cell line and primary FLSs established from a site of major inflammation in SpA. ICAM-1 expression is increased on the lining and sublining cells of the synovium and on adjacent endothelial cells in patients with SpA and is regulated by TNFα [39,40]. Our immunocytochemical staining for ICAM-1 showed a speckled pattern very similar to previous findings [35,36]. Carman and colleagues [35,36] revealed that the origin of this morphology is caused at least partly by the formation of ICAM-
1-enriched cell membrane protrusions. These protrusions are critical in the pro-active role of the endothelium in the transmigration of leukocytes [35,36]. The deposition of sCD11/CD18 complexes on the cells investigated in our experiment was not uniform and appeared in a way that could well reflect the selective binding to such ICAM-1-enriched zones. Indeed, the deposition was critically dependent on TNFα treatment of the cells, increasing the ICAM-1 expression. The requirement of a dense expression of ICAM-1 is furthermore consistent with the biochemical evidence reported earlier [18] that multimers of sCD11/CD18 (for example, 2 × CD11/CD18 and 4 × CD11/CD18) participate in a polyvalent interaction with ICAM-1 molecules, enabling a high avidity in the interaction [18,41]. In this way, our findings support that sCD11/CD18 selectively binds ICAM-1-rich features important for the interaction of leukocytes with the synovial lining and endothelium of blood vessels. Likewise, the clinical investigations made in our study suggest that insufficient saturation of these features with sCD11/CD18 captured from the plasma pool may aggravate inflammation, probably by permitting undue leukocyte adhesion.

Second, it is an important observation that the negative correlation between the decreased plasma level of sCD18 and disease activity in SpA could be simulated in cell cultures in vitro. Thus, CD18 shedding from SpA patient-derived PBMCs correlated negatively with the BASDAI score. This indicates that inflammation may be downregulated by the ability of leukocytes to deliver an elevated production of sCD18. In contrast, failure by the leukocytes to compensate the capture of sCD11/CD18 onto ICAM-1-expressing surfaces appears to augment inflammation. Taken together, these data also indicate that a compound resembling sCD18 could be beneficial as a therapeutic drug in SpA.

Our investigations identified monocytes as the predominant contributors to CD18 shedding among PBMCs in cultures in vitro. It is well established that the monocyte population can be separated into subsets with distinct regulatory capabilities [5,42,43]. Intermediate monocytes have both pro-inflammatory and anti-inflammatory functions. Thus, they are precursors of osteoclasts in psoriatic arthritis [7] and promote the expansion of the Th17 subset in RA [8]. However, intermediate monocytes also show the highest expression of the anti-inflammatory cytokine IL-10 and are tolerant to stimulation during infection [6,44]. Also, lack of CD16+ monocytes may be related to auto-inflammatory disease [45]. In line with previous studies, we found a high expression of CD18 integrins in the membrane of monocytes and especially the intermediate monocyte subset [46,47]. Furthermore, the concentration of sCD18 in PBMC supernatants correlated with the percentage of intermediate monocytes among all monocytes. This suggests that intermediate monocytes are the primary producers of sCD18 in the PBMC subset. In this way, our study proposes a novel role for intermediate monocytes in the blood as regulators of leukocyte migration. In SpA this regulatory role seems to be inadequate. Thus, in HLA B27-negative SpA only, the percentage of intermediate monocytes among all monocytes was increased, resulting in sufficient upregulation of CD18 shedding and low disease activity. However, this increase in intermediate monocytes was not seen in HLA B27-positive SpA, resulting in insufficient CD18 shedding and higher disease activity.

**Figure 7** Correlation between monocyte subsets and soluble CD18 (sCD18) in the peripheral blood mononuclear cell (PBMC) culture supernatant. The percentage of non-classic (A), intermediate (B), or classic (C) monocytes among all monocytes in a sample of PBMCs was correlated against the sCD18 concentration in supernatant from culture of the PBMCs. Samples were analyzed from PBMCs derived from a total of 5 healthy controls (HCs) and 10 patients with spondyloarthritis (SpA).
The high degree of overlap between plasma levels of sCD18 in patients with SpA and HCs implies that measuring sCD18 will not be of particular value in the diagnosis of SpA. However, measuring sCD18 could assist in discriminating between inflammatory activity and broader problems due to chronic changes in SpA. From a clinical viewpoint, discrimination between ongoing inflammation and chronicity is thus critical for choosing the proper clinical intervention [2]. To best measure the level of inflammation, many composite scores, such as the BASDAI, BASFI, and physician global assessment score, have been developed. However, these measures are only moderately objective and are difficult to compare across individuals. Also, BASDAI does not correlate well with objective measures such as CRP and MRI findings or erythrocyte sedimentation rate [48]. Nevertheless, in lieu of better measures of disease activity, the BASDAI is currently regarded as the gold standard regarding treatment initiation and response [49]. This underlines the need for better objective measures, especially because the role of CRP in SpA is debatable, as it is induced mainly by IL-6 and anti-IL-6 has little effect in SpA [50,51]. In agreement, CRP showed no correlations with self-assessment scores, clinical findings, or MRI activity scores in our study population (data not shown). By contrast, the decrease in plasma levels of sCD18 found here correlated with a number of single and composite inflammatory measures, including BASDAI, level of morning stiffness, and physician global assessment score and the objective measures BASMI and SII MRI activity score in patients with SpA. These associations were greater when adjusting for differences in age, disease duration, HLA-B27 status, and treatment at time of inclusion, making the study population more homogeneous. Additionally, no correlation was observed between sCD18 and MRI chronicity scores or patient global score. Although MRI is not considered the best measure of structural damage, the MRI chronicity scores used in this study were based on MRI features described previously to correlate with radiographic changes [30,31]. Thus, this adds to the validity of sCD18 as a marker of the inflammatory component of SpA disability. Importantly, the association with disease activity remained after correction for CRP.

Recently, the serum concentrations of both MMP-3 and MMP-9 have been found to be associated with disease activity in ankylosing spondylitis [32,53]. Shedding of CD18 occurs in processes that involve MMP-3 and MMP-9 [22,23]. With the observation that CD18 is a substrate for MMP-9 [23], measurement on sCD18 may provide an indication of enzymatic activity in vivo, which is central to pathogenic mechanisms of these diseases [25]. In this way, sCD18 could be a novel mechanistic biomarker of SpA disease activity involved in the disease pathogenesis and reflecting inflammatory processes [54,55].

No inflammatory disease controls were assessed in this study. Thus, the specificity of our findings in SpA is uncertain. Interestingly though, the plasma levels of sCD18 were decreased in plasma from HLA-B27-positive SpA patients compared with HLA-B27-negative patients. Additionally, shedding of CD18 from PBMCs was increased among HLA-B27-negative patients only. HLA-B27 positivity in patients with ankylosing spondylitis predicts a poorer prognosis and a better response to anti-TNFα treatment [56]. It is unclear whether this reflects a different pathogenesis in HLA-B27-positive versus HLA-B27-negative SpA. HLA-B27-positive SpA patients also had increased BASMI, CRP, and MRI activity scores compared with HLA-B27-negative SpA patients in our study population (data not shown). From the established correlations, the decreased levels of sCD18 in HLA-B27-positive SpA may thus be explained by the higher degree of disease activity in this group. However, mechanistic couplings between HLA-B27 and shedding of CD18 cannot be excluded. Validation studies are needed to clarify whether measuring the sCD18 plasma level can be used as a marker of inflammatory activity in SpA.

Conclusions

The clinical impact of CD18 deficiency has been long known from the several types of leukocyte adhesion deficiencies [57]. Our work now argues that more subtle changes in CD18 functions are sufficient to affect disease activity in SpA through alterations in the systemic concentration of sCD18. The influences appear to involve the failure of monocyte subsets to maintain a sufficient concentration of sCD11/CD18 complexes. Our work suggests that a delicate balance exists between cellular-expressed and sCD18 integrins, which may be disturbed by the well-characterized changes in MMP activity and ICAM-1 expression associated with a vast range of illnesses involving chronic inflammation. With the central role of CD18 integrins in supporting leukocyte migration and immunological synapse formation, it encourages the hypothesis that such disturbances are involved in the disease-causing mechanisms in SpA. This points to the level of sCD18 as a potential marker of inflammatory activity or a compound resembling sCD18 as a therapeutic drug.

Additional files

Additional file 1: Table S1. Associations at time of inclusion between plasma soluble CD18 (sCD18) levels in all patients with spondyloarthritis (SpA) and self-assessment scores after correction for age, disease duration, HLA-B27 status, treatment, and C-reactive protein (CRP).

Additional file 2: Table S2. Associations at time of inclusion between plasma soluble CD18 (sCD18) levels in all patients with spondyloarthritis (SpA) and clinical scores and test results after correction for age, disease duration, HLA-B27 status, treatment, and C-reactive protein (CRP).
Additional file 4: Figure S2. Depletion of soluble CD18 (sCD18) by binding to intercellular adhesion molecule 1 (ICAM-1) expressed on the human umbilical vein cell line EA.hy926 or spondyloarthritis (SpA) fibroblast-like synoviocytes (FLS). Culture medium supplemented with 50% (vol/vol) normal human serum (NHS) or 50% (vol/vol) synovial fluid mononuclear cell (SFMC) supernatant as sCD18 source were incubated with EA.hy926 or SpA FLS cells, each cell type either cultured in the supplemented media with 10 ng/mL tumor necrosis factor-alpha (TNFα) (to induce ICAM-1 expression) or in the supplemented media without further additions (Medium).

Supporting data

Additional file 3: Figure S1. Confocal microscopy analysis of the ability of sCD11/CD18 complexes to bind intercellular adhesion molecule 1 (ICAM-1) on the human umbilical vein cell line EA.hy926 or spondyloarthritis (SpA) fibroblast-like synoviocytes (FLSs). (A) Schematic of the cellular incubations. In step 1, adherent cells were incubated with 10 ng/mL tumor necrosis factor-alpha (TNFα), which increased the ICAM-1 expression. In step 2, a source of CD11/CD18 was added (that is, either NHS or supernatant of peripheral blood mononuclear cell (PBMC) culture). In step 3, biotinylated antibody recognizing ligand-binding activated CD11/CD18 (KIM127) was added followed by addition of fluorochrome-labelled streptavidin for detection with confocal microscopy. (B) Binding of soluble CD18 (sCD18) to TNFα-treated cells. As outlined above, in step 1, cells were treated with either TNFα or plain medium as a control. In step 2, either NHS or cell supernatant was used or plain medium was used as a control. In step 3, either the antibody to CD18 (KIM127) was used or biotinylated monoclonal IgG1 immunoglobulin was used as a control. Red staining indicated the binding of CD18, further indicated with white arrows. The positions of cell nuclei were located by 4′,6-diamidino-2-phenylindole (DAPI) staining, indicated in blue.

Abbreviations

BASDA: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Metrology Index; CR: complement receptor; CPT: C-reactive protein; DMEM: Dulbecco’s modified Eagle’s medium; FCS: fetal calf serum; FITC: fluorescein isothiocyanate; FLSs: fibroblast-like synoviocyte; HC: healthy control; ICAM-1: intercellular adhesion molecule 1; mAb: monoclonal antibody; MFI: mean fluorescence intensity; MMP: matrix metalloproteinase; MRI: magnetic resonance imaging; NHS: normal human serum; NK: natural killer; PBMC: peripheral blood mononuclear cell; RA: rheumatoid arthritis; RT: room temperature; sCD18: soluble CD18; SFMC: synovial fluid mononuclear cell; SIJ: sacroiliac joint; SpA: spondyloarthritis; TBS: Tris-buffered saline; TBS-T: elegy medium; TV-J: in classification.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

TW helped to design the study, to carry out the experiments, and to analyze and interpret the data and drafted the manuscript. TJ helped to design the study, to analyze and interpret the data and was involved in revising the manuscript. BD helped to design the study, to collect the patient samples and information, and to analyze and interpret the data and was involved in revising the manuscript. BJ and AK helped to carry out the experiments and to analyze and interpret the data and were involved in revising the manuscript. BM, RO, and WH helped to analyze and interpret the data and were involved in revising the manuscript. All authors read and approved the final manuscript.

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References

1. Dougados M, Baeten D. Spondyloarthritis. Lancet 2011, 377:2127–2137.
2. Braun J, van den Berg R, Baraliakos X, Boehm H, Burgos-Vargas R, Collantes-Estevez E, Dagfinrud H, Dikmans B, Dougdos M, Emery P, Geher P, Hammoduhed M, Inman RD, Jonkhees K, Khan MA, Kiitz U, Kien T, Leirisalo-Repo M, Makowskyj WP, Olivier I, Pavilia K, Sieper J, Stanislawiska-Biemart E, Wendling D, Ozopomen S, van Drogen C, van Royen B, van der Heijde D. 2010 update of the ASAS/EULAR recommendations for the management of ankylosing spondylitis. Ann Rheum Dis 2011, 70:985–994.
3. Makowskyj WP: Biomarkers in spondyloarthritis. Curr Rheumatol Rep 2010, 12:318–324.
4. Ambus J, Cerezczenko N, Tak K, Baeten D: Pathogenesis of spondyloarthritis: autoimmune or autoinflammatory? Curr Opin Rheumatol 2012, 24:351–358.
5. Ziegler-Heitbrock L, Hofer TP: Toward a refined definition of monocyte subsets. Front Immunol 2013, 4:23.
6. Horelt A, Belge KU, Steppich B, Pirioz J, Ziegler-Heitbrock L, The CD14 + CD16+ monocytes in eryispelas are expanded and show reduced cytokine production. Eur J Immunol 2002, 32:1319–1327.
7. Chu YC, Shao T, Feng C, Mensah KA, Thullen M, Schwarz EM, Ritchlin CT: CD16 (Fcgammall) as a potential marker of osteoclast precursor in psoriatic arthritis. Arthritis Res Ther 2010, 12:R14.
8. Rossol M, Kraus S, Peerer M, Baerwald C, Wagner U: The CD14(bright) CD16 + monocyte subset is expanded in rheumatoid arthritis and promotes expansion of the Th17 cell population. Arthritis Rheum 2012, 64:5671–5677.
9. Chata L, Sánchez-Arrià A, Pérez A, Cuende E, Albarrán F, Chevarria J, Sánchez MA, Monserratt I, de la Hera A, Prieto A, Sanz I, Diaz D, Alvarez-Mon M: Monocyte populations as markers of response to adalimumab plus MTX in rheumatoid arthritis. Arthritis Res Ther 2012, 14:R175.
10. Carman CV: Mechanisms for transcellular diapedesis: probing and pathfinding by ‘InVASive-like protrusions’. J Cell Sci 2009, 122:3025–3035.
11. Springer TA: Adhesion receptors of the immune system. Nature 1990, 346:425–434.
12. Lowen T, Straub RH: Integrins and their ligands in rheumatoid arthritis. Arthritis Res Ther 2011, 13:244.
13. Wilson RW, Ballantyne CM, Smith GW, Montgomery C, Bradley A, O’Brien WE, Beaudet AL: Gene targeting yields a CD14-mutant mouse for study of inflammation. J Immunol 1993, 151:1571–1578.
14. Wang H, Kess D, Lindqvist AK, Peters T, Sindriaru A, Wlaschek M, Balaytrry NA, Holmahl D, Schaarfechter-Kochanek K: A 9-9mermigon interval of chromosome 10 controls the T cell-dependent psoriasiform skin disease and arthritis in a murine psoriasis model. J Immunol 2008, 180:5520–5529.
15. Singh K, Gataza M, Peters T, Bohrner L, Hainzl A, Wang H, Sindriaru A, Schaarfechter-Kochanek K: Reduced CD18 Levels Drive Regulatory T Cell Conversion into Th17 Cells in the CD18hypo PlJ Mouse Model of Psoriasis. J Immunol 2013, 190:2544–2553.
16. Wang H, Peters T, Kess D, Sindriaru A, Oreshkova T, Van Rooyen N, Stratis A, Rentia AC, Sundelkerloto C, Wlaschek M, Haase I, Schaarfechter-Kochanek K: Activated macrophages secrete sphingosine-1-phosphate and sphingosine-1-phosphate mediated chronic psoriasiform skin inflammation. J Clin Invest 2006, 116:2105–2114.
17. Dunne JL, Ballantyne CM, Beaudet AL, Ley K: Control of leukocyte rolling velocity in TNF-alpha-induced inflammation by LFA-1 and Mac-1. Blood 2002, 99:336–341.
18. Geilstroop LC, Boesen T, Kragstrup TW, Jorgensen A, Klein NJ, Thiel S, Deleuran MW, Vorup-Jensen T: Shedding of large functionally active CD11/18.
CD18 Integrin complexes from leukocyte membranes during synovial inflammation distinguishes three types of arthritis through differential epitope exposure. J Immunol 2010, 185:4154–4168.

19. Evans BJ, McDowell A, Taylor PC, Hogg N, Haskard DO, Landis RC. Shedding of lymphocyte function-associated antigen-1 (LFA-1) in a human inflammatory response. Blood 2006, 107:3593–3599.

20. Shin HY, Simon S, Schmid-Schönbein GW. Fluid shear-induced activation and cleavage of CD18 during pseudopod retraction by human neutrophils. J Cell Physiol 2008, 214:528–536.

21. Zen K, Guo YL, Li LM, Bian Z, Zhang CY, Liu Y. Cleavage of the CD11b extracellular domain by the leukocyte serineproteases is critical for neutrophil detachment during chemotaxis. Blood 2011, 117:4895–4894.

22. Gomez IG, Tang J, Wilson CL, Yan W, Heinecke JW, Harlan JM, Raines EW. Metalloproteinase-mediated Shedding of Integrin beta2 promotes macrophage efflux from inflammatory sites. J Biol Chem 2012, 287:6581–6589.

23. Vaisar T, Kassim SY, Gomez IG, Green PS, Hargdoll P, Parkes W, Warshafsky CL, Raine JW, Heinecke JW. MMP-9 sheds the beta2 integrin subunit (CD18) from macrophages. Mol Cell Proteomics 2009, 8:1064–1069.

24. Ogata Y, Enghild JJ, Nagase H. Matrix metalloproteinase 3 (stromelysin) activates the precursor for the human matrix metalloproteinase 9. J Biol Chem 1992, 267:3581–3584.

25. Ram M, Sheer Y, Shoenefeld Y. Matrix metalloproteinase-9 and autoimmune diseases. J Clin Immunol 2006, 26:299–307.

26. Ahmed D, Shukla A, Pope RM, Stein-Ricarda M, Niedbala MJ. Expression of matrix metalloproteinase 9 (96-kd gelatinase B) in human rheumatoid arthritis. Arthritis Rheum 1996, 39:1576–1587.

27. Fraser A, Fearon U, Reece R, Emery P, Veale DJ. Matrix metalloproteinase 9, apoptosis, and vascular morphology in early arthritis. Arthritis Rheum 2001, 44:2024–2028.

28. Dougados M, van der Linden S, Juhlin R, Huitfeldt B, Amor B, Calin A, Cats O, Conner-Spady B, Palsat J, Lambert RG. Criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984, 27:361–368.

29. Madsen KB, Jurik AG. Magnetic resonance imaging grading system for active and chronic spondylarthritides changes in the sacroiliac joint. Arthritis Care Res (Hoboken) 2010, 62:111–118.

30. Madsen KB, Jurik AG. MRI grading method for active and chronic spinal changes in spondylarthritides. Clin Radiol 2010, 65:5–14.

31. Maksymowych WP, Inman RD, Salonen D, Dhillon SS, Krishnananthan R, Stone M, Conner-Spady B, Palats J, Lambert RG. Spondyloarthritides Research Consortium of Canada magnetic resonance imaging index for assessment of spinal inflammation in ankylosing spondylitis. Arthritis Rheum 2005, 53:502–509.

32. Maksymowych WP, Inman RD, Dhillon SS, Williams M, Stone M, Conner-Spady B, Palats J, Lambert RG. Spondyloarthritides research Consortium of Canada magnetic resonance imaging index for assessment of sacroiliac joint inflammation in ankylosing spondylitis. Arthritis Rheum 2005, 53:703–709.

33. Stebulis JA, Rossetti RG, Atez FJ, Zurier RB. SHU 557A: A transmigratory cup in leukocyte diapedesis. J Immunol 2008, 185:3559–3559.

34. Stebulis JA, Rossetti RG, Atez FJ, Zurier RB. SHU 557A: A transmigratory cup in leukocyte diapedesis. J Immunol 2008, 185:3559–3559.

35. Carman CV, Springer TA. A transmigratory cup in leukocyte diapedesis both through individual vascular endothelial cells and between them. J Cell Biol 2004, 167:377–388.

36. Carman CV, Jurik AG. Endothelial cells proactively form microvilli-like membrane projections upon intercellular adhesion molecule 1 engagement of leukocyte LFA-1. J Immunol 2003, 171:6135–6144.

37. Suchard SJ, Stetsko DK, Davis PM, Skala S, Paton D, Launay M, Dhar TG, Barish JC, Suslic V, Shuster DJ, Mclntyre KW, McKinnon M, Salters-Cid L. An LFA-1 (alphabeta2) small-molecule antagonist reduces inflammation and joint destruction in murine models of arthritis. J Immunol 2010, 184:3917–3926.

38. Feldmann M, Brennan FM, Maini RN. Rheumatoid arthritis. Cell 1996, 85:307–310.

39. Kruithof E, Baeten D, De Rycke L, Vandoorne B, Foell D, Roth J, Canete JD, Boots AW, Veys EM, De Keyser J. Synovial histopathology of psoriatic arthritis, both oligo- and polyarticular, resembles spondyloarthropathy more than it does rheumatoid arthritis. Arthritis Res Ther 2005, 7:R869–R850.

40. Shu Q, Amin MA, Ruth JH, Campbell PL, Koch AE. Suppression of endothelial cell activity by inhibition of TNFalpha. Arthritis Res Ther 2012, 14:R88.

41. Vonop-Jensen T. On the roles of polyvalent binding in immune recognition: perspectives in the nanoscience of immunology and the immune response to nanomedicines. Adv Drug Deliv Rev 2012, 64:1759–1781.

42. Ziegler-Heitbrock L, Ancuta P, Crowe S, Daiso M, Grau V, Hart DN, Leenen PJ, Liu YJ, MacPherson G, Gandolfi JG, Schreiber I, Schmitz J, Shottrman K, Sozanni S, Stroblo H, Zembaale M, Austyn JM, Lutz MB. Nomadulation of monocytes and dendritic cells in blood. Blood 2010, 116:784–860.

43. Ziegler-Heitbrock L. The CD14+ CD16+ blood monocytes: their role in inflammation and infection. J Leukoc Biol 2007, 81:589–592.

44. Skoczynska-Moncznik J, Bzowska M, Loseke S, Gagie-Giebnerow E, Zembaale M, Pyjama MM. Peripheral blood CD14High CD16+ monocytes are main producers of IL-10. Scand J Immunol 2008, 67:152–159.

45. Frankenberger M, Elsii AB, Angstwurm MW, Hoffmann H, Hofer TP, Heimbck M, Meyer P, Lohse P, Wist M, Haussinger K, Reis A, Ziegler-Heitbrock L. A defect of CD16-positive monocytes can occur without disease. Immunobiology 2013, 218:169–174.

46. Ancuta P, Rao R, Mosea A, Mehle A, Shay SK, Luskinas FW, Gobadza D, Fraktalkine preferentially mediates arrest and migration of CD16+ monocytes. J Exp Med 2003, 200:1701–1709.

47. Desroches CV, Andreani C, Ridal G. Differential expression of the LFA-1 molecule on the human peripheral blood mononuclear cell subpopulations. Immunol Lett 2000, 74:13–20.

48. Bredella MA, Steinbach LS, Morgan S, Ward M, Davis JC. MRI of the sacroiliac joints in patients with moderate to severe ankylosing spondylitis. AJR Am J Roentgenol 2006, 187:1420–1426.

49. van der Heijde D, Sieper J, Maksymowych WP, Dougados M, Burgos-Vargas R, Landewe R, Kuhle W, Braun J. Update of the international ASAS recommendations for the use of anti-TNF agents in patients with axial spondyloarthritis. Ann Rheum Dis 2010, 70:905–908.

50. Podubnyy DA, Raduljeva M, Listing J, Braun J, Sieper J. Comparison of a high sensitivity and standard C reactive protein measurement in patients with ankylosing spondylitis and non-axial radiographic axial spondyloarthritis. Ann Rheum Dis 2010, 69:1388–1411.

51. Schoels MM, van der Heijde D, Breedveld FC, Burmester GR, Dougados M, Emery P, Ferraccioli G, Gabay C, Gobfitsky A, Gomez-Reino JJ, Jones G, Kien TK, Murakami M, Nishimoto N, Smolen JS. Blocking the effects of interleukin-6 in rheumatoid arthritis and other inflammatory rheumatic diseases: systematic literature review and meta-analysis informing a consensus statement. Ann Rheum Dis 2013, 72:583–589.

52. Chen CH, Lin KC, Yu DT, Yang C, Huang F, Chen CH, Liang TH, Liao HT, Tsai CY, Wei JC, Chou CT. Serum matrix metalloproteinases and tissue inhibitors of metalloproteinases in ankylosing spondylitis: MMP-3 is a reproducibly sensitive and specific biomarker of disease activity. Rheumatology (Oxford) 2006, 45:414–420.

53. Mattey DL, Packham JC, Nikon NB, Coates L, Creamer P, Hailwood S, Taylor GJ, Bhalla AK. Association of cytokine and matrix metalloproteinase profiles with disease activity and function in spondyloarthritis. Ann Rheum Dis 2010, 70:909–913.

54. Kragstrup et al. Decreased plasma levels of soluble CD18 link leukocyte infiltration with disease activity in spondyloarthritis. Arthritis Research & Therapy 2014 16:R42.
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