Soluble macrophage-derived CD163 is a marker of disease activity and progression in early rheumatoid arthritis

S.R. Greisen¹, H.J. Møller², K. Stengaard-Pedersen³, M.L. Hetland⁴, K. Hørsløv-Petersen⁵, A. Jørgensen⁶, M. Hvid¹⁶, B. Deleuran¹,³

¹Institute of Medical Microbiology and Immunology, ²Department of Clinical Biochemistry and ³Rheumatology, ⁴Institute of Clinical Medicine, Aarhus University Hospital, Denmark; ⁵Department of Rheumatology, Glostrup Hospital, Denmark; ⁶Institute of Clinical Medicine, Aarhus University, Building 1240, Wilhelm Meyers Allé 4, DK-8000 Aarhus C, Denmark.

E-mail: b.deleuran@immunology.au.dk

Received on September 30, 2010; accepted on revised form on February 11, 2011.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2011.

Key words: CD163, arthritis, joint destruction, macrophages

ABSTRACT

Objective. To investigate the expression of the soluble form of the resident macrophage marker CD163 (sCD163) and its association with core parameters for disease activity, including radiographic progression in early rheumatoid arthritis (RA).

Methods. In a longitudinal sample set from early RA patients (n=34) we measured plasma levels of sCD163 at initiation of treatment and after 9 months of treatment compared with levels with disease activity in 28 joints (DAS28), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and total Sharp score (TSS). We also measured plasma levels of sCD163 in 55 healthy volunteers (HV) and in a transverse sample set of chronic (>8 years of disease) RA patients (n=24) and OA patients (n=24) with paired plasma and joint fluid.

Results. Early RA patients had significantly higher plasma levels of sCD163 (1.69mg/l (1.42–2.10)) (median (IQR)) at baseline than after 9 months of treatment (1.28mg/l (0.963–1.66), p=0.001), but not significantly changed compared with HV (1.66mg/l (1.22–2.02)). In early RA patients, baseline levels of sCD163, correlated with DAS28, CRP and ESR. Interestingly, sCD163 at 9 months was associated with radiographic progression (TSS) between year 0 and 5 (r=0.468, p=0.02). Levels of sCD163 were higher in RA patients, than in OA patients and higher in SF than in plasma.

Conclusion. Plasma levels of macrophage derived sCD163 are associated with disease activity and predict radiographic progression in early RA patients, supporting that sCD163 may have a role as a biomarker of disease activity and that resident macrophages are important for joint destruction.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory joint disease. Progression of the disease is associated with cartilage destruction and synovial inflammation, which subsequently result in malformation of the joint. Macrophages and fibroblasts represent the major producers of pro-inflammatory cytokines in the inflamed joint and are primarily present in the synovial lining, sub-lining layer and cartilage pannus junction (1). The infiltrating macrophages express high levels of HLA-DR, tumour necrosis factor α (TNF-α), interleukin (IL-) 1 and IL-6, all known contributors to inflammation (2). Thus macrophages play an important part in the progression of inflammation and joint destruction, supported by association between synovial macrophage infiltration and radiographic progression.

Macrophages in the joint are described as two subpopulations, M1 and M2 (3). M1 is believed to be the first macrophage at sites of inflammation. M2 macrophages are especially found resident in the joint and functionally attributed to both pro- and anti-inflammatory activity (4-6). M2 is characterised by elevated expression of the scavenger receptor CD163.

CD163 is expressed by macrophages and strongly regulated by external stimuli (7). IL-10, IL-6 and glucocorticoids upregulate CD163 expression, while lipopolysaccharide (LPS), TNF-α and interferon γ (IFN-γ) result in downregulation of its surface expression (8).

CD163 is also found in a soluble form (sCD163) due to ectodomain shedding by TNF-α converting enzyme (TACE), which is also responsible for the release of TNF-α (9). The soluble form is upregulated in plasma during acute inflammation such as sepsis, but also during chronic inflammation as in RA (10). Plasma levels of sCD163 have previously been examined in chronic RA and spondylitis (11, 12).

In this study we investigated the association between sCD163 and core parameters for disease activity as well as joint destruction in early RA patients.

Materials and methods

Collection of samples

A longitudinal set of plasma samples was obtained from the CIMESTRA study, with demographic data listed in Table I (14). The patients were newly diagnosed RA patients, with symptoms for no longer than six months, all corticosteroid naïve at the time of entry (n=34). Plasma samples were...
BRIEF PAPER

Soluble macrophage-derived CD163 in early RA / S.R. Greisen et al.

obtained from treatment initiation (day 0) and after 9 months of treatment. The study was a double blinded randomised study, where patients at entry were randomised to conventional methotrexate (MTX) treatment combined with an aggressive regime of intraarticular betamethasone injections, with (n=18) or without (n=16) cyclosporine. The two groups are considered as one since no difference in treatment response after 5 years was observed in the current study, in line with the original study (14). All plasma samples were collected at the outpatient clinic of Aarhus University Hospital. Clinical data were obtained the same day as collection of plasma samples. Radiographic measurements were obtained the day of treatment initiation and again 2, 3, 4 and 5 years after diagnosis. Radiographic scoring was done using total Sharp score (TSS).

In this study we made use of disease activity in 28 joints (DAS28), Health Assessment Questionnaire (HAQ), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) all recorded in the CIMESTRA study. At the time of diagnosis, the median DAS28 was 5.2 (4.4–5.9), decreasing to 1.9 (1.4–2.4) at 9 months, indicating a high level of disease activity at the time of diagnosis, and low disease activity after 9 months. A cross-sectional sample set of plasma and synovial fluid (SF) was obtained from chronic RA patients (n=24) at the time of knee arthrocentesis, all with disease duration of 8 years or more (Table I). Patients received classical DMARDs only and prednisolone (n=2). A cross sectional sample set of plasma and SF was also obtained from osteoarthritis (OA) patients (n=24). Plasma samples were obtained from healthy volunteers (HV), age- and gender- matched with the CIMESTRA patients (n=55), (age 56 years (46–64) vs. 58 years (52–69) p=0.20; gender 62% vs. 65% women, respectively). All plasma and SF samples were collected in heparinised tubes and kept at -80°C until used. All samples were obtained after informed written consent according to the Danish Data Protection Agency, the Local Ethics Committee (project numbers 20050046 and 20060012) and the Declaration of Helsinki.

Table I. Baseline demographic, clinical and serologic data from patients with, early rheumatoid arthritis (RA), chronic RA, osteoarthritis (OA) and healthy volunteers.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Early RA</th>
<th>Chronic RA</th>
<th>OA</th>
<th>Healthy volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>34</td>
<td>24</td>
<td>24</td>
<td>55</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58 (52–69)</td>
<td>61 (56–71)</td>
<td>68 (62–80)</td>
<td>56 (46–64)</td>
</tr>
<tr>
<td>Gender (% females)</td>
<td>65%</td>
<td>75%</td>
<td>79%</td>
<td>70%</td>
</tr>
<tr>
<td>MTX (No.)</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MTX +CyA (No.)</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other DMARD (No.)</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DAS28</td>
<td>5.2 (4.4–5.9)</td>
<td>NA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RF positive (%)</td>
<td>65%</td>
<td>79%</td>
<td>62%</td>
<td>62%</td>
</tr>
<tr>
<td>Anti-CCP positive (%)</td>
<td>60%</td>
<td>62%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>18.9 (7.8–42.1)</td>
<td>48 (16.5–70.0)</td>
<td>All &lt; 8</td>
<td>All &lt; 8</td>
</tr>
<tr>
<td>ESR (mm)</td>
<td>19.5 (10.0–44.8)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>TSS</td>
<td>3.0 (0.0–3.9)</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
</tbody>
</table>

Values are expressed as medians with interquartile ranges in parentheses. Treatment and clinical data included: MTX: methotrexate, CyA: cyclosporine A, Other DMARD: hydroxychloroquine, sulfasalazine, and prednisolone, DAS28: disease activity score in 28 joints, RF: rheumatoid factor, anti-CCP: anti-cyclic citrullinated peptide antibody, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, TSS: total Sharp score, NA: not assessed. Early RA were all corticosteroid naïve and with symptoms less than 6 months. Chronic RA patients all had a minimum of 8 years disease and samples were taken at the time of knee arthrocentesis.

Fig. 1. Plasma (PL) and synovial fluid (SF) levels of sCD163 in healthy volunteers (HV) (n=55), early rheumatoid arthritis (early RA) (n=34), measured at initiation of treatment (0 month) and after 9 months of treatment (9 months), in patients with more than 8 years of disease (chronic RA) (n=24) and in patients with osteoarthritis (OA) (n=24). Bars represent median, IQR and 5-95 percentiles, with the level of significance indicated by *p<0.01, **p<0.001 and ***p<0.0001.

Fig. 2. Plasma levels of sCD163 measured in patients at initiation of treatment (0 month) versus after 9 months of treatment, showing a linear association, with dotted lines indicating 5% and 95% level of confidence interval.
Soluble macrophage-derived CD163 in early RA / S.R. Greisen et al.

Table II. In patients with early rheumatoid arthritis, plasma levels of sCD163 after 9 months of treatment correlated with change in disease progression (TSS) between year 0 and 5, whereas DAS28 did not (*p<0.05).

<table>
<thead>
<tr>
<th></th>
<th>sCD163</th>
<th>sCD163</th>
<th>DAS28</th>
<th>DAS28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 month</td>
<td>9 months</td>
<td>0 month</td>
<td>9 months</td>
</tr>
<tr>
<td>Change in TSS from year 0 – 3</td>
<td>0.177 (0.36)</td>
<td>0.258 (0.18)</td>
<td>-0.358 (0.057)</td>
<td>-0.260 (0.18)</td>
</tr>
<tr>
<td>Change in TSS from year 0 – 4</td>
<td>-0.07 (0.75)</td>
<td>0.260 (0.23)</td>
<td>-0.286 (0.19)</td>
<td>-0.239 (0.28)</td>
</tr>
<tr>
<td>Change in TSS from year 0 – 5</td>
<td>0.312 (0.12)</td>
<td>0.468 (0.018)*</td>
<td>0.005 (0.98)</td>
<td>-0.067 (0.75)</td>
</tr>
</tbody>
</table>

ELISA
sCD163 was measured in sandwich ELISA essentially described in detail (15).

Statistics
Statistical analyses were performed using GraphPad Prism 5.0 for Mac (GraphPad Software, La Jolla, CA). All graphic data are expressed as medians with interquartile ranges (IQR) and 5 to 95 percentiles. All data in text are expressed as medians with IQR in parentheses. Grouped analyses were done by the Kruskal-Wallis test with Dunn’s multiple comparison test as a post-hoc analysis. Evaluation of paired samples was done by Wilcoxon’s matched paired test, whereas non-paired data were examined by Mann-Whitney U-test. Correlation was tested using Spearman’s Rho. In all tests the level of significance was a two-sided p-value of less than 0.05.

Results
Plasma levels of sCD163 are elevated in chronic RA, but not in early RA
In the longitudinal sample set of newly diagnosed RA patients we measured plasma levels of sCD163 at baseline (1.69 mg/l (1.42–2.10)) and after 9 months of treatment (1.28 mg/l (0.963–1.66)) (Fig. 1). Plasma levels of sCD163 were significantly higher at baseline than after 9 months of treatment, and a strong linear association between plasma levels at baseline and after 9 months was observed (Fig. 2). The sCD163 plasma levels were not increased at the time of diagnosis compared with HV (1.66 mg/l (1.22–2.02)) whereas treatment resulted in significantly lower sCD163 levels compared to HV (Fig. 1).
Plasma levels of sCD163 in chronic RA patients were also measured and found significantly increased (3.05 mg/l (1.84–6.04) p<0.001) (Fig. 1), with sCD163 levels in SF elevated to 8.32 mg/l (6.04–10.65). Levels of sCD163 in SF and plasma were associated (r=0.4, p=0.05). Plasma and SF levels of sCD163 in chronic RA patients were also found significantly higher than in OA (plasma (2.07 mg/l (1.78–2.59)) and SF (3.44 mg/l (2.59–4.60)), which again were found increased compared with HV (Fig. 1).

Plasma levels of sCD163 are associated with parameters for disease activity and disease progression in early RA
In early RA patients, plasma levels of sCD163 correlated to CRP (r=0.357, p=0.038), ESR (r=0.536, p=0.0018) and DAS28 (r=0.464, p=0.0057) at baseline. After 9 months of treatment correlation was still observed to CRP (r=0.375, p=0.034) and ESR (r=0.627, p<0.001). We also examined for association between plasma level of sCD163 and change in radiographic score (Delta TSS) revealing a strong association between radiographic progression from year 0 to 5 and sCD163 at 9 months (r=0.468, p=0.018) whereas no association to DAS28 was observed (Table II).

Discussion
In this study we demonstrate that in patients newly diagnosed with RA, plasma levels of sCD163 reflects both disease activity and radiographic progression. Our results suggest that if the immunosuppression is not adequate within 9 months in early RA, this is associated with disease progression, as measured by delta TSS at 5 years.
CD163 is solemnly expressed by macrophages and is cleaved by TACE, which also cleaves membrane bound TNF-α, IL-6R and several other bioactive peptides. However, clearance of these substances often occurs rap-

Conclusion
...
References


