Concise Report

MHC class II expression on myeloid cells inversely correlates with disease progression in early rheumatoid arthritis

R. B. Mueller¹,², A. Skapenko¹,², J. Wendler³, F. Schuch³, J. R. Kalden² and H. Schulze-Koops¹,²,⁴

Objective. To investigate whether MHC class II expression on myeloid cells of patients with treatment-naive early rheumatoid arthritis (RA) correlates with disease progression.

Methods. Monocytes were isolated by negative selection from the peripheral blood of 15 patients with early RA (disease duration ≤12 months), differentiated to macrophages and analysed for MHC class II expression by flowcytometry. The phenotypical data were correlated with clinical disease progression for up to 45 months.

Results. Before treatment was initiated, in vitro differentiated macrophages of 10/15 early RA patients expressed MHC class II comparable with macrophages from healthy controls. In sharp contrast, macrophages of the remaining five patients expressed significantly fewer MHC class II molecules. In contrast to patients with normal levels of myeloid cell MHC class II expression, who developed a smouldering, non-progressive disease, patients with decreased expression of MHC class II on macrophages early in their disease developed a continuously active disease as demonstrated by persistently increased disease activity scores ($\chi^2 = 4.54$, $P < 0.02$) and progressive bone destructions ($\chi^2 = 5.66$, $P < 0.02$) despite aggressive therapy.

Conclusion. The level of myeloid cell MHC class II expression in recent onset RA allows a reliable distinction between patients who develop active and destructive RA and patients with a smouldering, slowly progressive disease.

Key words: Rheumatoid arthritis, Monocytes, Macrophages.

Introduction

Current evidence suggests that early treatment of rheumatoid arthritis (RA) significantly improves the prognosis of the disease [1]. However, as the course of RA is variable [2], it is of importance to identify RA patients who are likely to suffer from a severe, progressive and destructive disease and patients with a slow, smouldering disease in order to individually adapt the anti-inflammatory therapy and justify costs as well as the risk of severe treatment-related side effects.

Among various other factors, the degree of systemic inflammation has been identified as a reliable predictor of disease progression in early RA [3] suggesting that persistent systemic inflammation may drive the disease to a tissue-destructive course irrespective of anti-inflammatory treatment. We could recently show that the expression of major histocompatibility complex (MHC) class II molecules on peripheral blood-derived myeloid cells of patients with RA inversely correlates with disease activity and that alterations of disease activity, e.g. initiated by anti-inflammatory therapy, reflect on the expression of MHC class II on myeloid cells [4]. These observations lead us to analyse whether MHC class II expression on myeloid cells from the peripheral blood of patients with early, treatment-naive RA, reflects ongoing disease activity related to systemic inflammation and allows to predict clinical disease progression.

Patients and methods

Patients

The patient population consisted of 15 patients with recent-onset RA (<12 months of clinical symptoms) who had never been treated with disease modifying anti-rheumatic drugs (DMARDs) or corticosteroids. At the time of first analysis, all patients had active disease as indicated by increased serum levels of CRP, increased ESR and elevated DAS28 levels (Table 1). Eighteen age- and sex-matched healthy donors served as controls (Table 1). The study was approved by the University of Erlangen-Nuremberg Institutional Review Board and written informed consent was obtained from all individuals before they entered the study.

Monocyte isolation and macrophage differentiation

Monocytes were isolated from peripheral blood mononuclear cells by negative selection employing mAbs labelled with magnetic beads following the instructions from the manufacturer (Miltenyi Biotec, Bergisch-Gladbach, Germany). Recovered cells were more than 90% pure for CD14⁺ monocytes among all viable cells in all experiments. To induce macrophage differentiation, monocytes were cultured on plastic dishes as described [4].

Flowcytometry

Myeloid cells (1 × 10⁵/sample) were stained before, during and after differentiation to macrophages with saturating amounts of fluorescein-isothiocyanate-conjugated anti-HLA-DR mAb (Dako Diagnostika, Hamburg, Germany) and analysed by FACS (EPICS, Beckman Coulter, Cambridge, MA, USA).

Results

Patients with early active RA were analysed for MHC class II expression on peripheral blood monocytes and in vitro differentiated macrophages upon their first presentation to a
rheumatoid. The data revealed a considerable variation of myeloid MHC class II expression. However, no significant differences in the mean expression level and the distribution of MHC class II levels between RA patients and healthy controls were detected (Fig. 1A).

To analyse whether MHC class II expression on myeloid cells at disease onset correlated with the subsequent course of the disease, patients were monitored for up to 45 months after their initial visit. Therapy was managed with corticosteroids and methotrexate based on the clinical status at the discretion of a rheumatologist who was unaware of the study questions. Two groups of patients could be identified according to their clinical disease activity at the end of the follow-up: patients (n = 10) who had at least a moderate response to treatment (reduction of DAS28 ≥ 0.6 [5]) and patients (n = 5) with continuously active or even aggravated disease (stable or increased DAS28; Fig. 1B). One year after onset of the disease, those RA patients who responded well to treatment at the end of the follow-up displayed a significant decrease of the DAS28 from 5.2 ± 1.3 to 2.6 ± 1.3, whereas patients with active disease at the end of the follow-up presented also with elevated inflammatory activity at 1yr after disease onset (DAS28 = 4.5 ± 0.9) (Fig. 1C; P = 0.006). To further analyse disease progression in the two groups, standard radiographs were taken from inflamed joints and evaluated by an independent rheumatologist for the presence of joint erosions [6]. All patients with elevated DAS28 had developed bone erosions at the end of the follow-up period, whereas only 2/10 patients with a treatment-induced decrease of the DAS28 presented with bone erosions (Fig. 1D, P < 0.004). Of note, the two groups were similar for demographical data (age and sex distribution), and no significant differences were found for disease duration, conventional parameters of disease activity (CRP, ESR) at the beginning of the disease, or the medication employed (Table 1).

In marked contrast, when myeloid cell MHC class II expression at the first visit to a rheumatologist (Fig. 1A) was associated with the development of a progressive or non-progressive disease in individual patients, it became apparent that monocytes of treatment-naive, early RA patients with a poor subsequent clinical outcome had a significantly decreased ability to up-regulate MHC class II expression during macrophage differentiation compared with monocytes from patients with a favourable clinical outcome and compared with healthy control donors (P < 0.001, Fig. 1E).

### Table 1. Demographic data of the study population

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Non-progressive disease</th>
<th>Progressive disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>15</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>54.3  (±13.3)</td>
<td>53 (±13.4)</td>
<td>58 (±12.1)</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>12/3</td>
<td>8/2</td>
<td>4/1</td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>5.3   (±3.3)</td>
<td>4.8 (±2.3)</td>
<td>6.8 (±4.6)</td>
</tr>
<tr>
<td>Follow up (months)</td>
<td>24.9  (±15.3)</td>
<td>24.7 (±16.6)</td>
<td>25.2 (±12.7)</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>29.8  (±26)</td>
<td>28.3 (±21.2)</td>
<td>32.8 (±33.3)</td>
</tr>
<tr>
<td>RF (pos/neg)</td>
<td>11/4</td>
<td>6/4</td>
<td>5/0</td>
</tr>
<tr>
<td>DAS 28 (base line)</td>
<td>5.1   (±1.6)</td>
<td>5.2 (±1.3)</td>
<td>4.8 (±1.9)</td>
</tr>
<tr>
<td>Medication (corticosteroids/MTX/other)</td>
<td>15/12/2</td>
<td>10/7/1</td>
<td>5/5/1</td>
</tr>
<tr>
<td>Change of DMARD treatment</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

ESR: erythrocyte sedimentation rate; RF: rheumatoid factor; DAS: disease activity score; DMARD: disease-modifying antirheumatic drug.

**Fig. 1.** Myeloid cell MHC class II expression inversely correlates with continuously active, erosive disease. (A) HLA-DR expression (mean fluorescence intensity, MFI) on freshly isolated monocytes (left panel) and on in vitro differentiated macrophages (right panel) from individual early RA patients are demonstrated as dots. The bars indicate mean MFI of cells from 18 controls. (B) DAS28 changes in individual patients from initial evaluation to end of follow-up. Left panel: patients with at least a moderate decrease of disease activity; right panel: patients with continuously active or progressive disease. Moderate and significant changes in disease activity are represented by dark and light grey areas, respectively. (C) DAS28 levels (mean ± S.D.) 1yr (±3 months) after disease onset. (D) Bone erosions: patients with or without bone erosions (as assessed by standard radiographs) are represented by dark and light grey bars, respectively. (E) Mean (±S.D.) HLA-DR expression of myeloid cells from RA patients with non-progressive disease (n = 10, squares) and RA patients with progressive disease (n = 5, triangles). Controls are depicted as diamonds with a grey shadow representing the 95% confidence interval. Insert: HLA-DR expression on freshly isolated monocytes of patients with progressive or non-progressive disease. Differences in data distribution were calculated by two-tailed Student’s t-test (B, C) or chi-squared analysis (D).
Moreover, when the patients were stratified according to the ability of their myeloid cells to express MHC class II at the time of the initial diagnosis of the disease, a reduced ability to express MHC class II was significantly associated with the subsequent development of persistently active disease ($\chi^2 = 4.54$, $P < 0.02$) and with the occurrence of joint erosions ($\chi^2 = 5.66$, $P < 0.02$). Consequently, reduced myeloid cell MHC class II expression at early stages of the disease conferred a relative risk to develop persistently active disease and bone erosions of 3.5 and 4.5, respectively, to treatment-naive early RA patients.

Discussion

In this pilot study, we demonstrate that myeloid cell MHC class II expression in recent onset, treatment-naive RA is inversely correlated with the intensity of subsequent disease progression. Early RA patients with reduced myeloid cell MHC class II expression at disease onset had a 3.5- and 4.5-fold increased risk to develop an erosive or a continuously active disease, respectively, when compared with patients with myeloid cell MHC class II expression in the range of healthy controls. Our study, therefore, identifies myeloid cell MHC class II expression at disease onset as a potential novel predictive marker for the development of progressive, erosive RA.

Despite the necessity for predicting disease progression in early RA, reliable factors are sparse and their values are incompletely defined. Rheumatoid factor, disease activity at the time of diagnosis of the disease [7], the shared epitope [8], C reactive protein [9], synovial cell infiltration [10] and most recently, an IL-4 receptor single nucleotide polymorphism [11] have been suggested to be useful for the determination of the future course of the disease. Among those factors, clinical disease activity at the time of diagnosis has most frequently been attributed with correctly anticipating disease outcome [3], although its value in very early disease has not been firmly established. Disease activity in RA is associated with and mediated by increased levels of pro-inflammatory cytokines, such as TNF and IFN-γ. As MHC class II expression on myeloid cells is regulated by TNF and IFN-γ [4], the data suggest that MHC class II expression on myeloid cells might be a function of disease activity in RA. We have previously demonstrated, that MHC class II expression on myeloid cells from patients with established RA is inversely correlated with disease activity [4]. The finding of the current study, that reduced MHC class II expression on myeloid cells at the time of disease diagnosis in treatment-naive early RA patients appears to predict the course of the disease more reliably than conventional markers of disease activity (CRP, ESR, DAS28) suggests that reduced MHC class II expression on myeloid cells is an early abnormality induced by pro-inflammatory cytokines in RA and, thus, appears to be more sensitive with regard to assessing disease activity compared with the conventional markers in early disease. Of note, because of the pilot nature of our study, the data do not allow precise determination of the predictive value of reduced myeloid cell MHC class II expression in comparison with RF-positivity or antibodies to citrullinated peptides (anti-CCP). The frequencies of RF-positive and RF-negative patients within the group of patients with progressive and non-progressive disease (Table 1) were not significantly different and uniform serum anti-CCP antibody levels were not available in the study patients as different anti-CCP ELISA were used during the time of the recruitment of the patients, and in some patients anti-CCP ELISA had not been performed at the time of the first analysis.

Together, our data suggest that myeloid MHC class II expression in treatment-naive early RA patients may allow to predict the subsequent clinical course of the disease and, therefore, might have a potential value as a novel, very early, yet reliable prognostic factor in early disease.

Rheumatology key message

- The level of myeloid cell MHC class II expression in early RA may allow distinction between patients who develop active destructive RA and patients with a slowly progressive disease.

Acknowledgements

This study was supported by the Deutsche Forschungsgemeinschaft (Schu 756/2-3, and 2-4), the German Ministry for Education and Research (Network for Competence Rheumatology, project C2-5), and the Interdisciplinary Center for Clinical Research at the University Hospital of the University of Erlangen-Nuremberg (Project B27 and grant to R.B. Mueller).

The authors have declared no conflicts of interest.

References