Increased expression of Siglec-1 on peripheral blood monocytes and its role in mononuclear cell reactivity to autoantigen in rheumatoid arthritis

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Abstract

Objectives. Elevated expression of Siglec-1 on circulating monocytes has been reported in some inflammatory and autoimmune diseases, but its expression and role in RA has not been elucidated. The aims of this study were to determine the expression of Siglec-1 in peripheral blood and to explore its role in mononuclear cell reactivity to autoantigen in RA.

Methods. Siglec-1 protein and mRNA levels in 42 RA patients, 39 OA patients, 28 SLE patients and 42 normal controls were determined by flow cytometry and quantitative RT-PCR, respectively. In addition, 10 patients with active RA received DMARDs for 12 weeks and the frequencies of Siglec-1-positive cells and the 28-joint DAS (DAS28) were assessed before and after therapy. Furthermore, TNF-α, IFN-γ and type II collagen were used to up-regulate Siglec-1. Peripheral blood mononuclear cells (PBMCs) from different groups were stimulated with mitogens or antigens and cell proliferation and cytokine production were determined.

Results. The protein and mRNA levels of Siglec-1 on PBMCs and monocytes in RA patients were significantly higher than those in OA patients and healthy controls. Moreover, the expression of Siglec-1 protein on PBMCs was positively correlated with DAS28, ESR, high-sensitivity CRP and IgM-RF, but not with anti-CCP antibody. Interestingly, Siglec-1 expression was decreased in parallel with the decrease in the DAS28 after 12 weeks of anti-rheumatic treatment. Furthermore, TNF-α, IFN-γ and type II collagen can up-regulate Siglec-1 in PBMCs. Elevated PBMC proliferation and proinflammatory cytokine production to collagen stimulation in RA patients decreased when Siglec-1 was inhibited by anti-Siglec-1 antibodies.

Conclusion. Elevated Siglec-1 expression in PBMCs and monocytes can potentially serve as a biomarker for monitoring disease activity in RA. Siglec-1 may also play a proinflammatory role in stimulating lymphocyte proliferation and activation in RA.

Key words: Siglec-1, biological markers, RA, disease activity score, proliferation, activation.

Introduction

RA is a chronic, systemic autoimmune disease characterized by articular synovitis. It occurs worldwide, with male and female incidence ratios of 1:2 to 1:4 and the prevalence in adults is ~1%. However, different races and ethnic groups have different morbidities, which reflect susceptibility genes such as HLA-DR4 in the onset of RA. Recurrence or persistence of synovitis can lead to articular cartilage and bone destruction, joint dysfunction or disability, and the quality of life of these patients is seriously affected. A small number of patients have symptoms beyond articulus, such as rheumatoid nodules and...
lung diseases. Although the pathogenesis of RA has not yet been clarified, innate immunity mediated by monocytes–macrophages has been implicated to play an important role in the development of RA [1–3].

Siglec-1 is a member of the sialic acid–binding immunoglobulin-like lectins (Siglecs) family, which is thought to promote cell–cell interactions and regulate the functions of cells in the innate and adaptive immune systems through glycan recognition [4]. Human Siglec-1 was first cloned by Paul R. Crocker’s group and was expressed exclusively in tissue-resident macrophages [5]. Evidence is accumulating that Siglecs, particularly Siglec-1, are important players in the initiation and progression of inflammatory and autoimmune diseases. A previous study found that Siglec-1 was expressed on inflammatory macrophages of rheumatoid synovial membranes [5]. But little is known about whether Siglec-1 is expressed on circulating monocytes of RA patients and what the exact role of Siglec-1 is in systemic inflammation of RA. As an important biomarker of monocyte–macrophage activation and a type I IFN-specific imprint, Siglec-1 may internalize collagen-associated antigens and present them to T cells or autoreactive B cells. Siglec-1-positive macrophages in synovial fluid may also interact with synovial fibroblast to induce proinflammatory cytokine production and inflammatory response initiation in the joint cavity. Therefore Siglec-1 may provide a potential pathophysiological link between antigen presentation, autoreactive lymphocytes activation, inflammation and RA.

Accordingly, we explored whether Siglec-1 is highly expressed on circulating monocytes and, if it is, what the possible role of Siglec-1 is in mononuclear cell reactivity to autoantigen in RA. First, Siglec-1 protein and mRNA levels in 42 RA patients, 39 OA patients, 28 SLE patients and 42 normal controls were determined by flow cytometry and quantitative RT-PCR, respectively. Second, the 28-joint DAS (DAS28), ESR, serum high-sensitivity CRP (hs-CRP), IgM-RF and anti-CCP antibody were determined in RA patients and correlated to Siglec-1 protein levels. Third, 10 patients with active RA received DMARDs of MTX plus HCQ for 12 weeks and the frequencies of Siglec-1-positive cells and the DAS28 were assessed before and after therapy. Fourth, TNF-α, IFN-γ and type II collagen were used to up-regulate Siglec-1. Also, peripheral blood mononuclear cells (PBMCs) from different groups were stimulated with mitogens or antigens and cell proliferation and cytokine production were determined.

Materials and methods

Subjects

The study conforms to the principles outlined in the Declaration of Helsinki and was approved by the Bioethics Committee of Chengdu Military General Hospital, Chengdu, China. After informed consent was obtained from the participants, 42 RA patients who were diagnosed according to the ACR 1987 revised criteria for RA [6] were enrolled in the study. Age- and sex-matched patients (39 OA, 28 SLE) and 42 healthy volunteers were also recruited. SLE patients were diagnosed according to the ACR criteria [7] and all SLE patients had active disease, with a mean SLEDAI score of 15.3 (range 8–24). All of the controls had no evidence of swollen or tender joints and were both RF and anti-CCP antibody negative. People with evidence of infectious disease, immunological disorders, fever, anti-inflammatory treatment, recent major surgery or neoplastic disease were excluded from the study. A detailed description of the study participants is provided in Table 1. A blood sample from each participant was drawn by clean venepuncture of an antecubital vein after an overnight fast.

Fluorescence-activated cell sorting

PBMCs from 10 ml of whole blood were prepared as below and residual erythrocytes were lysed at 4°C with EL buffer (Qiagen, Hilden, Germany). The cells were then washed twice with PBA (PBS + 0.5% BSA + 0.05% sodium azide). A total of 2 × 10⁶ cells in 100 μl PBA were first Fc-blocked with 2 μg of human IgG for 15 min at room temperature and subsequently incubated with antibody for Siglec-1 (HSn 7D2) (Abcam, Cambridge, UK) at a concentration of 10 μg/ml for 1 h. After washing, the cells were resuspended in 100 μl PBA and stained with 549 conjugated goat anti-mouse secondary antibody (Jackson ImmunoResearch, West Grove, PA, USA) at a concentration of 5 μg/ml for 30 min. Mouse IgG (2 μg) was used to block the free valencies of the secondary antibody before being stained with 20 μl FITC-conjugated mouse anti-human CD14 antibody (eBioscience, San Diego, CA) and phycoerythrin-Cy7 conjugated mouse anti-human CD16 antibody (BD, Franklin Lakes, NJ, USA) for 30 min at room temperature in the dark. The cells were then washed and resuspended in 500 μl PBS. Dead cells were excluded by adding 5 μl of Dead Cell Discriminator (Clonetics, USA) per 10⁶ cells directly to the cell suspension immediately prior to flow cytometric analysis. Cells were analysed using an FC500 flow cytometer and CXP Analysis Software (Beckman Coulter, Fullerton, CA, USA). The laser and optical filter configuration used to detect FITC and 549 conjugated goat anti-mouse secondary antibody were 488/525 and 488/575 nm, respectively. The FL4 channel (675 nm) was used to exclude DCD-positive cells. Cell debris was excluded on forward scatter (FS) and side scatter (SS) plots with gating to eliminate the lower FS signal with variation of SS signals. Appropriate isotype-matched control antibodies were used in parallel.

Monocyte preparation and total RNA extraction

PBMCs were isolated from participants by standard Ficoll density-gradient centrifugation (Lymphoprep; Axis-Shield PoC, Oslo, Norway) at 800 g for 25 min. CD14⁺ monocytes were purified from PBMCs by positive selection using CD14 MicroBeads (Miltenyi Biotec, Bergisch-Gladbach, Germany). RNA isolation was performed using the RNeasy Mini kit (Qiagen) according to the manufacturer’s instructions. To avoid genomic DNA contamination, DNA degradation was performed using RQ1 RNase-Free DNase.
All results are given as mean (s.d.) unless otherwise noted. There were no significant differences between groups by ANOVA (for means) or chi-square (for sex ratio). NA, not applicable.

**TABLE 1** Baseline clinical data for the four study groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>RA (n = 42)</th>
<th>OA (n = 39)</th>
<th>Control (n = 42)</th>
<th>SLE (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female, n</td>
<td>7/35</td>
<td>6/33</td>
<td>8/34</td>
<td>5/23</td>
</tr>
<tr>
<td>Age, years</td>
<td>48.3 (12.5)</td>
<td>45.7 (10.6)</td>
<td>47.2 (9.3)</td>
<td>46.6 (11.4)</td>
</tr>
<tr>
<td>Duration of RA, years</td>
<td>8.3 (6.9)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Morning stiffness, hours</td>
<td>1.22 (0.87)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Number of swollen joints</td>
<td>7.5 (8.6)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Number of tender joints</td>
<td>4.5 (4.8)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>DAS28</td>
<td>4.13 (1.37)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>&lt;2.6, n (%)</td>
<td>8 (19.0)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2.6–3.2, n (%)</td>
<td>2 (4.8)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>3.2–5.1, n (%)</td>
<td>21 (50)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>&gt;5.1, n (%)</td>
<td>11 (26.2)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.52 (3.56)</td>
<td>26.13 (3.68)</td>
<td>25.32 (2.45)</td>
<td>25.87 (3.15)</td>
</tr>
<tr>
<td>Current use of DMARDs or TNF-α inhibitor, n (%)</td>
<td>36 (85.7)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

All results are given as mean (s.d.) unless otherwise noted. There were no significant differences between groups by ANOVA (for means) or chi-square (for sex ratio). NA, not applicable.

Quantitative RT-PCR

PCR analysis was performed as described previously [8,9]. Briefly, cDNAs were synthesized by using the SuperScript III First-Strand Synthesis kit (Invitrogen, Carlsbad, CA, USA) with oligo dT primers. Primers for Siglec-1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were designed with Primer Express software, version 3.0 (Applied Biosystems, Foster City, CA, USA) and verified to generate a single product specific to target genes by the BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast/). Primers were as follows: human Siglec-1 (GenBank NM_023068.3), sense primer 5’-GGC TGTAGCATGGTTATGATGT-3’, antisense primer 5’-AA TCAAGGGCATCTTTAGGGATA-3’, amplicon size 82 bp; human GAPDH (GenBank NM_002046.3), sense primer 5’-CCATCAATGACCCCTTCATTG-3’, antisense primer 5’-CATGGTGGAATCATATTGGAAC-3’, amplicon size 66 bp. Real-time PCRs were performed using the ABI 7500 Sequence Detection System with SYBR Green PCR Master Mix (Applied Biosystems). Specificity of the products was confirmed by agarose gel electrophoresis. As a control for cross-contamination, samples consisting of distilled water were also subjected to the isolation procedures and the extracts were tested with all assays. Cycle threshold (Ct) values were calculated after confirming similar amplification efficiencies of target and endogenous control cDNA and gene expression was analysed using the ∆∆Ct method [10].

Determination of ESR, serum hs-CRP, IgM-RF and anti-CCP antibody

The level of serum hs-CRP and IgM-RF were determined by latex-enhanced nephelometric immunoassay (Dade-Behring BN II, Marburg, Germany). IgM-RF values >15 IU/ml were considered positive. Anti-CCP antibody was detected by Diastat Anti-CCP (Axis-Shield, Dundee, UK) with the recommended cut-off value of 5 U/ml. ESR was also measured.

Assessment of DAS28

The DAS28 was calculated using the following complex formula, which includes the number of tender (t28) and swollen (s28) joints, ESR and global health on a visual analogue scale (VAS) [11]:

\[
\text{DAS28} = 0.56 \times \sqrt{t28} + 0.28 \times \sqrt{s28} + 0.7 \times \ln(\text{ESR}) + 0.014 \times \text{VAS}.
\]

The disease activity was evaluated by the DAS28 according to the following scale: low activity, 2.6–3.2; moderate activity, 3.2–5.1; high activity, >5.1.

Stimulation of PBMCs with TNF-α, IFN-γ and type II collagen

PBMCs of healthy controls were plated in 24-well plates at a concentration of 2 × 10⁵ cells/well in 2 ml complete media (RPMI 1640 supplemented with 10% fetal bovine serum, 20 mM l-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin). Cells were stimulated with one of the following reagents: TNF-α (5 ng/ml), IFN-γ (5 ng/ml) or type II collagen (12.5, 25 or 50 μg/ml). PBMCs were harvested after 48 h of stimulation for analysis of Siglec-1 expression by FACS as described above.

Mononuclear cell proliferation and cytokine production

The experiment was performed as described previously, with minor modifications [12,13]. The PBMCs of RA and OA patients and healthy controls were washed twice with PBS and cultured in complete RPMI 1640 medium (Gibco, Grand Island, NY, USA). In some cases, blocking antibody against Siglec-1 (2 μg/ml, HSn 7D2, Santa Cruz Biotechnology, Dallas, TX, USA) was used to block the expression of Siglec-1 functionally. The cells were then
plated at a density of $2 \times 10^5$ cells/well (200 μl) in triplicate in 96-well flat-bottomed plates, with or without the following antigens or mitogens: phytohaemagglutinin (PHA) 10 μg/ml, ovalbumin 50 μg/ml, bovine type II collagen 50 μg/ml (Sigma, St Louis, MO, USA). The cells were incubated for 3 days at 37°C in 5% carbon dioxide and culture supernatants were collected for cytokine detection. Cells were then cultured for another 2 days. Before the last 6 h of culture, 0.5 μCi of [3H]thymidine was added to each well. Cells were harvested onto nitrocellulose and the radioactivity incorporated was counted in a scintillation counter. Lymphocyte responses were expressed as stimulation indices (SIs), defined as the ratio of cpm in the presence of antigens to cpm without antigens.

ELISA for cytokines

TNF-α and IFN-γ were measured in the culture supernatants with ELISA kits according to the manufacturer’s specifications (R&D Systems, Minneapolis, MN, USA). The limits of detection of the assays performed were 15.6–1000 pg/ml for TNF-α and IFN-γ. When required, samples were diluted before testing.

Statistical analysis

For normally distributed data, an unpaired t-test was performed to test for differences in means between two groups and analysis of variance (ANOVA) for more than two groups. For non-normally distributed data, significance analyses between more than two groups was performed using the Kruskal-Wallis H-test, while significance between two groups was analysed using the Mann-Whitney U-test. The correlation of two different parameters was calculated with the Spearman rank order correlation. P-values < 0.05 were considered significant.

Results

Increased expression of Siglec-1 protein on PBMCs in RA patients

As shown in Fig. 1, the rate of Siglec-1-positive cells in the total PBMCs in the RA group was significantly higher than in the OA group and normal controls, but there was no difference between the OA group and the control group. Furthermore, the Siglec-1-positive rate in SLE patients was similar to that in RA patients (with $P > 0.05$). Most of the Siglec-1-positive cells also expressed the inflammatory monocyte marker CD14, however, some CD14low cells also expressed Siglec-1, which may belong to the CD14lowCD16+ resident monocyte subset (Fig. 1D). A similar result was also found in SLE patients in another study [14]. Moreover, the mean fluorescence intensity (MFI) of Siglec-1 was also higher in the RA group than in the OA group and healthy controls, but the MFI in SLE patients was higher than in RA patients.

Increased expression of Siglec-1 mRNA on monocytes of RA patients

For mRNA detection, PCR amplification curves and melt curves were used (see supplementary Fig. S1, available at Rheumatology Online). Statistical results showed that the
normalized Siglec-1 mRNA level in RA patients was significantly higher than in OA patients and control individuals (Fig. 1H), but no significant difference was observed between OA patients and normal controls. Furthermore, the relative Siglec-1 mRNA level in SLE patients was higher than in RA patients.

Siglec-1 expression is positively correlated with DAS28, ESR, IgM-RF and hs-CRP, but not with anti-CCP antibody

When correlative studies were performed, positive correlations between the Siglec-1 protein level and DAS28, ESR, IgM-RF and hs-CRP were observed ($r = 0.84, 0.71, 0.50$ and $0.74$, respectively; all $P < 0.001$) (see Fig. 2). However, no linear correlation between the Siglec-1 protein level and anti-CCP antibody was found ($r = 0.15, P = 0.35$; Fig. 2C).

Decreased Siglec-1 expression on PBMCs after anti-rheumatic treatment

As shown in Fig. 3A and B, the mean rate of Siglec-1-positive PBMCs decreased from $20.42\%$ (s.d. $4.30$) to $7.07\%$ (s.d. $1.87$) ($P < 0.001$ by paired t-test) after therapy. Meanwhile, the DAS28 decreased from $5.27$ (s.d. $0.80$) to $3.25$ (s.d. $1.35$) ($P < 0.001$).

#### Fig. 2 Correlative study of Siglec-1 with the DAS28, ESR, hs-CRP, IgM-RF and anti-CCP antibodies.

In RA patients, a positive correlation between Siglec-1-positive cells in total PBMCs (%) and (A) hs-CRP, (B) IgM-RF, (D) ESR and (E) the DAS28 were observed. However, no correlation between the Siglec-1 protein level and (C) anti-CCP antibodies was found. $n = 42$ for each plot.
The Siglec-1-positive rate for both the inflammatory monocyte [from 61.78% (S.D. 27.87) to 31.26% (S.D. 14.53)] and resident monocyte [from 35.77 (S.D. 16.89) to 19.13 (S.D. 10.87)] subsets were decreased after therapy (Fig. 3D). The decrease in Siglec-1 frequencies on PBMCs and on both the inflammatory and resident monocyte subsets reflected remission after therapy and may serve as a potential biomarker for monitoring anti-rheumatic therapy in RA.

**Discussion**

As a major biomarker of macrophage activation, Siglec-1 (sialoadhesin, Sn, CD169) is mainly expressed on tissue resident macrophages, but it can also be expressed on circulating monocytes under inflammatory conditions. Biesen et al. [14] found that Siglec-1 expression in inflammatory and resident monocytes is a potential biomarker for monitoring disease activity and the success of therapy in SLE. York et al. [15] found that Siglec-1 was up-regulated on PBMCs in SSC patients and type I IFN and certain Toll-like receptor (TLR) agonists, including TLR7 and TLR9, and can induce Siglec-1 mRNA and protein expression in vitro. Ikezumi et al. [16] identified Siglec-1-positive cells as a macrophage subset whose accumulation in the kidney correlates with proteinuria and histologic damage in proliferative glomerulonephritis. Asano et al. [17] demonstrated that Siglec-1-positive macrophages such as lymph node-resident antigen-presenting cells dominating early activation of tumour antigen-specific CD8+ T cells by cross-presenting dead cell-associated antigens. All these data together suggest that Siglec-1 has a proinflammatory function on circulating monocytes or tissue macrophages and Siglec-1 is important in modulating T cell function and activation during immune responses.
Although other studies have elucidated the importance of Siglec-1 in inflammatory and autoimmune diseases, this is the first study to explore whether Siglec-1 is expressed on circulating monocytes and whether its expression is correlated with disease activity in RA. We found that the Siglec-1 protein and mRNA levels were significantly increased in RA patients compared with OA patients and normal controls. And the increased expression of Siglec-1 protein was positively correlated with DAS28, ESR, hs-CRP and IgM-RF, but not with anti-CCP antibody. Moreover, Siglec-1 expression on PBMCs and on different monocyte subsets was decreased in parallel with the decrease in the DAS28 after anti-rheumatic treatment. These findings demonstrate that circulating monocytes in RA patients have been activated and that Siglec-1 on monocytes may serve as a potential biomarker for monitoring disease activity in RA.

Our results and previous studies also revealed that overexpression of Siglec-1 may be attributed to proinflammatory cytokine stimulation, such as TNF-α, IFN-γ, IFN-α, IL-1 etc. [5, 15, 18]. More importantly, as a major autoantigen in the pathogenesis of RA, type II collagen can also up-regulate monocyte expression of Siglec-1. Activated monocytes may phagocytose more collagen through surface Siglec-1 and process and present it to autoreactive T and B cells in the periphery blood and joint cavity. Inhibition of Siglec-1 expression on RA PBMCs can decrease lymphocyte proliferation and reduce TNF-α and IFN-γ secretions, which will further down-regulate Siglec-1 expression on monocytes and limit the inflammatory process of RA [19]. On the other hand, uncontrolled proinflammatory cytokine production in RA patients will activate monocytes, increase Siglec-1 expression and promote lymphocyte proliferation and TNF-α and IFN-γ secretions, eventually generating a positive feedback on monocytes and lymphocytes and exacerbating the inflammation of RA.

Another study identified a type I IFN signature in peripheral blood cells from a subgroup of RA patients [20]. Our previous study also verified Siglec-1 as an imprint of type I IFN activation and a risk biomarker for monitoring disease severity in atherosclerosis [9]. It is well known that the associated inflammation and coronary atherosclerosis is elevated in RA patients [21-23]. So both coronary artery atherosclerosis and RA are activated by the type I IFN system, and Siglec-1 may provide a direct link between the two diseases.

There are two major human monocyte subsets: CD14+CD16− inflammatory monocytes and CD14low CD16+ resident monocytes [24-26]. Our results showed that Siglec-1 was highly expressed on CD14+ monocytes in RA patients. Some CD14low cells can also express Siglec-1, which may belong to the CD14lowCD16+ subset. Inflammatory monocytes are key players in the
first line of host defence against pathogens, including bacteria and viruses. Microbes and their components may activate host immunity and enhance type I IFN secretion, thus resulting in greater expression of Siglec-1 in RA patients. Ancuta et al. [27] showed that resident monocytes were more likely to differentiate into dendritic cells than inflammatory monocytes. So whether resident monocytes can present autoantigens in a Siglec-1-dependent manner in RA still needs to be clarified.

The DAS was developed in the early 1990s [28, 29] and later it was transformed into the DAS28 [11] in an era when therapy with biologicals was not yet available. However, Vander Cruyssen et al. [30] found that the DAS28 was an important variable for evaluating insufficient response to infliximab therapy and that this variable can be only slightly improved by adding supplemental variables. Moreover, the DAS28 has been considered the most important indicator for monitoring disease activity in RA. In our study, a strong correlation between Siglec-1 and the DAS28 was found, which indicated that Siglec-1 on monocytes may serve as a potential biomarker for monitoring disease activity in RA. Since the DAS28 is calculated using several parameters and is somewhat inconvenient for clinicians, measurement of Siglec-1 may provide a fast and accurate method for monitoring disease activity, pre-treatment assessment and therapeutic evaluation.

CRP is the most useful biochemical marker for the evaluation of disease activity in patients with RA [31]. McConkey et al. [32] revealed that CRP, which reflects exacerbations and remissions of RA, responds to changes in clinical activity more closely than the ESR. In contrast, another study demonstrated that the association between ESR and the DAS28 was closer than that between CRP and the DAS28 [33]. Our present study found that both ESR and CRP were positively correlated with the DAS28 (data not shown). Furthermore, Siglec-1 expression on PBMCs was positively correlated with both hs-CRP and ESR in RA patients. So we can conclude that Siglec-1 is also a useful biomarker for monitoring disease activity in RA.

RF and anti-CCP antibodies are B cell activation biomarkers in RA. Sellam et al. [34] found that RF or anti-CCP antibodies and elevated IgG are two simple biomarkers that can be used routinely before therapy to predict response to rituximab in patients with refractory RA. Clavel et al. [35] found that disease-specific ACPA-containing immune complexes induced dose-dependent TNF-α secretion by macrophages via engagement of FcγRIIa. Our study revealed a correlation between monocyte Siglec-1 and serum IgM-RF levels, but not with anti-CCP antibody, which indicated Siglec-1 may also be used for monitoring B cell activation and predicting response to therapy in RA patients.

In summary, the mRNA and protein levels of Siglec-1 on monocytes and PBMCs in patients with RA were significantly increased compared with OA patients and healthy individuals. Most importantly, the expression of Siglec-1 was positively correlated with the DAS28, ESR, hs-CRP
and IgM-RF, which indicates that Siglec-1 on PBMCs may serve as a potential biomarker for monitoring disease activity in RA. Moreover, TNF-α, IFN-γ, and type II collagen can up-regulate Siglec-1 on PBMCs. Elevated PBMC proliferation and proinflammatory cytokine production due to stimulation by collagen in RA patients decreased when Siglec-1 was inhibited by anti-Siglec-1 antibody, but its other roles in the pathogenesis of RA still need to be clarified.

**Rheumatology key messages**

- Siglec-1 was increased and positively correlated with the DAS28, ESR, hs-CRP and IgM-RF in RA.
- TNF-α, IFN-γ and type II collagen can up-regulate Siglec-1 in peripheral blood mononuclear cells.
- Elevated peripheral blood mononuclear cell proliferation and activation by collagen was partly due to Siglec-1.

**Acknowledgements**

We thank all the RA, OA and healthy participants in this study. We also thank Dr Ling-Zhen Zhang and Dr Hao Wang for their technical support and constructive suggestions.

**Funding**: This work was supported by the National Natural Science Foundation of China (81072479, 81170263) and the Hospital Management Fund of Chengdu Military General Hospital (to Y.S.X.).

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

**Supplementary data**

Supplementary data are available at Rheumatology Online.

**References**


