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Soluble erythropoietin receptor levels associate with inflammatory mediators but not with disease activity or cumulative organ damage in patients with systemic lupus erythematosus

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Abstract

The erythropoietin receptor (EpoR) stimulates erythrocyte proliferation after erythropoietin binding. EpoR belongs to the cytokine receptor superfamily and can be found on macrophages and endothelial cells. As there are no data on the role of EpoR systemic autoimmune diseases, we investigated the role of soluble EpoR (sEpoR) in patients with systemic lupus erythematosus (SLE). In a cross-sectional study we recorded clinical characteristics, disease activity (SLEDAI-2K) and organ damage (SDI). sEpoR, autoantibodies and cytokines were measured by enzyme-linked immunosorbent assay (ELISA) in SLE patients (n = 100) and compared with a rheumatoid arthritis (RA) cohort (n = 57) and a cohort with non-inflammatory back pain (NIBP; n = 89). Data were analysed with non-parametric techniques. We found no significant difference in sEpoR levels across the SLE, RA and NIBP groups and sEpoR levels were similar in patients with (6% of SLE and 31% of RA) or without anaemia. sEpoR levels were unrelated to haemoglobin levels, SLEDAI-2K or SDI scores, but in both cohorts correlated with levels for C-reactive protein (CRP), interleukin-6 (IL-6), tumour necrosis factor (TNF) and IL-1 (all $P < 0.001$). sEpoR levels are not involved in anaemia or erythropoietin resistance in SLE and RA patients, but closely mirror the underlying inflammatory process. This suggests that increased shedding of sEpoR during inflammation occurs at other sites than bone marrow.

Keywords

anaemia, anaemia of chronic disease, cytokine receptor, disease activity, erythropoietin, erythropoietin receptor, inflammation, rheumatoid arthritis, systemic lupus erythematosus

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Introduction

Systemic lupus erythematosus (SLE) is a pleomorphic autoimmune disease in which up to 60% of patients experience anaemia of chronic disease (ACD) despite appropriate levels of erythropoietin (EPO).[1] The erythropoietin receptor (EpoR) is expressed by erythroid cells but are also present in the brain, endothelium and on macrophages, suggesting that EpoR activation can exert extra-haematopoietic functions.[2,3] In SLE, EpoR may

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thus contribute to cellular activation and proliferation in vascular, renal, CNS and synovial tissue.[4–6] Where EpoR is a membrane-bound heterodimer, alternative splicing produces a soluble form of EpoR (sEpoR) which can be detected in human blood.[7]

Few studies have investigated the role of sEpoR. Baynes et al.[8] found that the presence of sEpoR correlated with enhanced erythropoiesis. In contrast, Yoshida et al.[9] detected no difference in sEpoR levels across healthy and anaemic patients, nor any correlation with haemoglobin, reticulocytes or EPO. Other studies have described a correlation between sEpoR and inflammatory mediators including interleukin-6 (IL-6) and tumour necrosis factor (TNF).[10]

As these findings offer limited insight into the role of sEpoR in rheumatic disease, we compared sEpoR levels between SLE patients, rheumatoid arthritis (RA) patients and non-inflammatory controls, and investigated in-depth whether sEpoR levels associated with anaemia, autoantibody and cytokine levels, and the presence and severity of clinical disease activity in SLE.

Methods

In a cross-sectional study of 100 patients who fulfilled the American College of Rheumatology's (ACR) classification criteria for SLE, we obtained informed consent, clinical data and blood samples during an outpatient visit. Disease activity was measured with the SLE Disease Activity Index-2K (SLEDAI-2K)[11] with active disease defined as SLEDAI-2K$\geqslant$3. The SLICC Damage Index (SDI) was used to quantify organ damage.

Serology

sEpoR levels were measured in 100 µL aliquots of serum stored at −20ºC using commercially available solid phase sandwich enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions (Duo set EpoR, R&D systems, MN, USA). Absorbance was read with a microplate reader using 450 nm as the primary wavelength and 600 nm as the reference wavelength to generate a four-parameter logistic standard curve. The samples were assayed in duplicate, with the result being the average of the two. Assay range is 2.5–4000 pg/mL with CV of 4.2% with no confounding by EPO addition. Non-detectable levels sEpoR (occurring in n=35) were assigned the limit of detection (LOD) value of 1 pg/mL for computation purposes.

Comparator groups consisted of HLA-B27 negative patients with non-inflammatory back pain (NIBP, n=89) and RA patients (n=57) all fulfilling ACR criteria.

Anti-dsDNA and other autoantibody assays were performed at a clinical immunology laboratory. Cytokines were measured by a quantitative sandwich immunoassay (Single Analyte ELISArrayTM kit; SuperArray Bioscience Corp., Frederick, MD, USA). The manufacturer's recommendations were followed throughout: the same lot was used for each cytokine, all assays were run in duplicate and the results were averaged. For statistical purposes values below the LOD were replaced by the LOD value (1 pg/mL).

Statistical analysis

Data are presented as measure of central tendency, that is, median with interquartile range, mean with standard deviation, or count and percentage. Anaemia was defined as Hb $<$ 13.0 g/dL for males and $<$11.5 g/dL for females with iron deficiency defined by concurrent ferritin levels $<$15 µg/L and ACD by ferritin $>$50 µg/L. Differences between groups were assessed with either t-test, non-parametric Mann–Whitney U-test or Chi-square test. Correlation coefficients (Rs) are derived from a Spearman's rho correlation test. Statistical significance was set at $\alpha=0.05$ and analyses performed on IBM SPSS Version 24.0.

Results

SLE, RA and NIBP patients had similar overall sEpoR levels (Figure 1). Six SLE patients (6%) were anaemic: one had iron deficiency anaemia (IDA), one had autoimmune haemolytic anaemia (AIHA) and the remaining four patients had ACD. In contrast, 21 RA patients (36%) were anaemic; three (5.3%) had IDA and 18 (31.7%) had ACD, while four NIBP patients (4.5%) were anaemic. Although sEpoR levels were slightly higher in anaemic patients in all the three cohorts, this did not reach statistical significance (all $P>0.3$; Figure 2).

sEpoR levels did not associate with SLEDAI-2K (Figure 3), SDI or use/dosage of prednisolone or immunosuppressive drugs, but sEpoR inversely

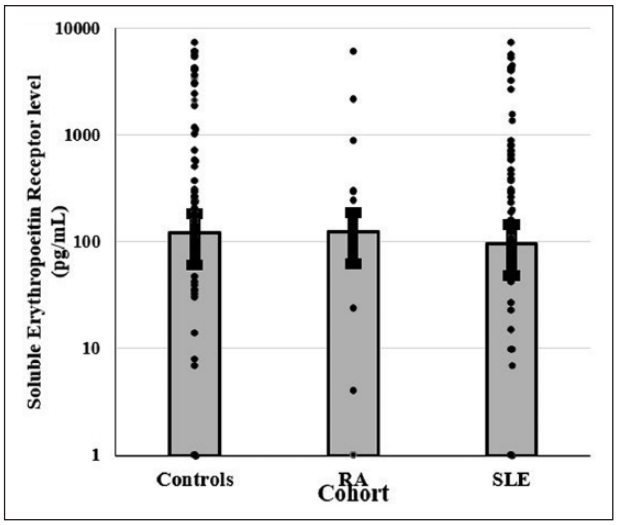

Figure 1. Soluble erythropoietin receptor levels for SLE patients (n = 100), RA patients (n = 57) and non-inflammatory back pain patients (controls; n = 89). *Y*-axis shows log scale for sEpoR levels and bars indicate interquartile range (25%–75%) with horizontal line indicating median levels. *P* value derived from Mann–Whitney U test, $P = 0.73$.

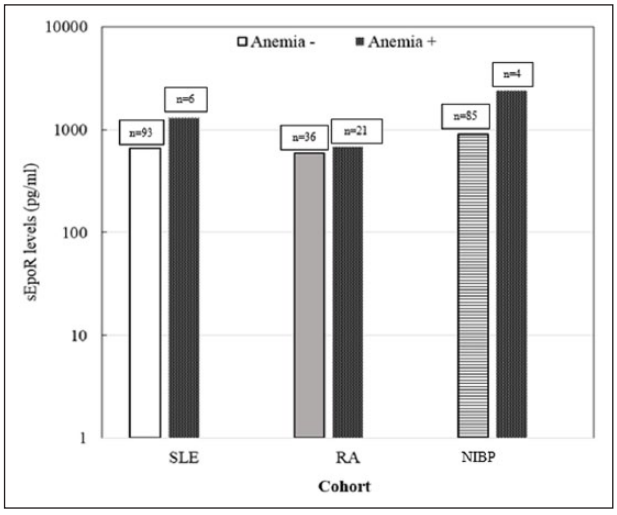

Figure 2. Median soluble erythropoietin receptor levels in the absence or presence of anaemia for SLE patients (n = 100), RA patients (n = 57) and NIBP patients (n = 89). *P* values were >0.3 for comparison between anaemic and non-anaemic individuals in all the three cohorts.

correlated with proteinuria (Rs −0.21, $P = 0.041$), discoid lesions (Rs −0.21, $P = 0.040$) and Raynaud's phenomenon (Rs −0.31, $P = 0.003$). By contrast, in RA patients sEpoR levels positively correlated with swollen/tender joint counts (Rs 0.26, $P = 0.009$).

sEpoR levels did not correlate with anti-dsDNA antibody levels or the presence of anti-ENA

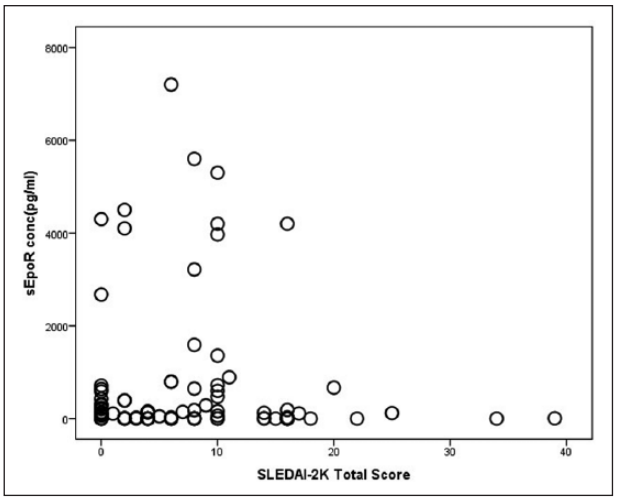

Figure 3. Scatterplot showing the absence of association between sEpoR and SLE disease activity as measured by SLEDAI-2K. Rs −0.087, $P = 0.409$.

antibodies (Table 1), but correlated with levels for C-reactive protein (CRP; Rs 0.28, $P = 0.007$) and a range of proinflammatory cytokines: interferon gamma (IFN-γ; Rs 0.35, $P = 0.001$), IL-1β (Rs 0.33, $P = 0.001$), IL-4 (Rs 0.33, $P = 0.001$), IL-6 (Rs 0.44, $P < 0.001$), IL-17A (Rs 0.26, $P = 0.011$), macrophage inflammatory protein (MIP)-1α (Rs 0.62, $P < 0.001$) and TNF-α (Rs 0.25, $P = 0.018$). However, sEpoR levels did not correlate with B-cell activity factor (BAFF) levels, regardless of the presence of anaemia (data not shown). In RA patients sEpoR levels correlated with erythrocyte sedimentation rate (ESR; Rs 0.29, $P < 0.01$), IL-1β (Rs 0.32, $P < 0.011$) and TNF-α (Rs 0.43 $P < 0.029$) levels, but not with IL-6 (Rs 0.01, $P = 0.90$). Inflammatory cytokines IFN-γ, IL-4, IL-17A and MIP-1α were not measured in RA patients.

Discussion

In this first report on sEpoR levels in rheumatic disease cohorts, sEpoR levels were comparable across SLE, RA and NIBP patients. With no relation to the presence or type of anaemia, our data support that sEpoR does not reflect EPO deficiency nor antagonise EpoR in SLE or RA,[9] contrary to speculations of previous studies.[10,12] While the low prevalence of anaemia may have confounded a possible association between sEpoR and anaemia in SLE, we observed a similar lack of association in RA patients where anaemia prevalence was 36%. sEpoR levels correlated with active joint

Table 1. Association between soluble erythropoietin receptor levels and markers of inflammation in SLE patients. Figures reflect nr (%) or median (IQR).

Biomarker	Median (IQR) or n (%)	Rs	*P* value
Haemoglobin (g/L)	13.1 (12.0, 14.05)	−0.025	0.813
Iron	13.0 (10.0, 17.5)	−0.026	0.814
Ferritin	78 (37, 161)	0.05	0.650
ESR	20 (10, 34)	0.031	0.773
CRP	4 (3, 4)	0.282	**0.007**
Creatinine	61 (52, 70)	−0.138	0.193
Platelets	255 (212, 296)	−0.004	0.968
Anti-dsDNA Ab (ELISA)	12 (0.0, 75)	0.115	0.271
Anti-RibP Ab titre (n < 11)	2.9 (2.1, 5.6)	0.034	0.770
Anti-Nucl Ab titre (n < 20)	45 (9, 198)	0.058	0.623
Anti-C1q Ab titre (n < 11)	1.5 (0.7, 4.5)	0.019	0.874
Anti-SSA Ab pos.	36 (37.1%)	0.033	0.756
Anti-RNP Ab pos.	18 (18.9%)	−0.067	0.538
Anti-Sm Ab pos.	7 (7.4%)	−0.077	0.475
BAFF (pg/mL)	1.74 (1.29, 2.35)	−0.064	0.543
IFN-γ (pg/mL)	62.49 (19.60, 134.07)	0.353	**0.001**
IL-1β (pg/mL)	17.90 (17.90, 17.90)	0.334	**0.001**
IL-4 (pg/mL)	7.0 (7.0, 7.0)	0.331	**0.001**
IL-6 (pg/mL)	14.00 (14.00, 19.53)	0.439	**<0.001**
IL-10 (pg/mL)	5.90 (5.90, 22.04)	0.177	0.092
IL-12 (pg/mL)	24.63 (12.6, 61.73)	0.177	0.092
IL-17 (pg/mL)	28.4 (28.4, 63.46)	0.264	**0.011**
MCP-1 (pg/mL)	133.71 (78.73, 219.83)	0.049	0.642
MIP-1α (pg/mL)	15.00 (15.00, 103.54)	0.616	**<0.001**
MIP-1β (pg/mL)	204.29 (161.17, 292.54)	0.180	0.086
TNF-α (pg/mL)	34.27 (21.40, 87.43)	0.246	**0.018**
TGF-1β (pg/mL)	592.34 (347.09, 859.68)	0.035	0.737

SLE: systemic lupus erythematosus; IQR: interquartile range; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; ELISA: enzyme-linked immunosorbent assay; Ab: antibodies; BAFF: B-cell activity factor; IFN-γ: interferon gamma; IL: interleukin (1β–17); MCP: monocyte chemoattractant; MIP: macrophage inflammatory protein; TNF: tumour necrosis factor; TGF: transforming growth factor. P values in bold indicate results of statistical significance ($p<0.05$).

counts in RA patients but did not correlate with clinical or serological markers of disease activity in SLE patients. In contrast, sEpoR levels correlated strongly with acute phase reactants and proinflammatory cytokines (IFN-γ, IL-1β, IL-4, IL-6, IL-17, MIP-1α and TNF-α) in SLE, supporting reports of sEpoR induction by IL-6 and TNF.[10]

These findings make it unlikely that the increased sEpoR during inflammation results from shedding by immune or erythroid cells. Further investigation is required to determine the site of sEpoR production. The limitations of this study include the lack of longitudinal sEpoR data and the exclusive Caucasian make-up of the study cohorts, while determination of Epo, anti-Epo and anti-EpoR antibody levels would be a useful complement to understanding of sEpoR in future studies.

In conclusion, we found no relation for sEpoR with anaemia nor with clinical or serological disease activity in SLE. As sEpoR closely follows markers of inflammation, this suggests a yet undefined role for sEpoR in the inflammatory response, but erythroid and immune cells are unlikely to be the source of increased sEpoR shedding.

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