Interleukin-10 receptor expression in systemic lupus erythematosus and rheumatoid arthritis

A.P. Cairns, A.D. Crockard¹, A.L. Bell

Queen’s University Musculoskeletal Education and Research Unit, Department of Rheumatology, Musgrave Park Hospital, Belfast; and ¹Regional Immunology Service, Royal Group of Hospitals, Belfast, Northern Ireland, UK.

Andrew P. Cairns, MD, MRCP, Specialist Registrar; Alistair D. Crockard, PhD, FRCPath, Consultant Clinical Scientist; Aubrey L. Bell, MD, FRCP, Senior Lecturer and Consultant Rheumatologist.

This work was supported by Lupus UK Merker and Consultant Rheumatologist. A.B. Bell, MD, FRCP, Senior Lecturer and Consultant Rheumatologist.

Key words: Systemic lupus erythematosus, interleukin-10 receptor, rheumatoid arthritis.

ABSTRACT

Objective. We aimed to determine the expression of the interleukin-10 receptor (IL10R) on circulating leukocytes in SLE and rheumatoid arthritis, and correlate this with plasma IL-10 levels and disease activity.

Methods. Peripheral blood was sampled from 20 SLE patients, 14 rheumatoid arthritis patients, and 14 healthy controls. IL-10R expression was determined by immunofluorescence labelling and flow cytometric analysis. Plasma IL-10 levels were measured by ELISA.

Results. IL-10R was highly expressed on monocytes, and to a lesser degree on neutrophils in all 3 patient groups. Only a small percentage of lymphocytes expressed IL-10R in all three groups. There was no significant difference in IL-10R expression on the surface of monocytes, neutrophils or lymphocytes in any of the 3 groups. IL-10R expression did not correlate with plasma IL-10 levels or disease activity.

Conclusion. This study has shown no difference in surface IL-10R expression between SLE, rheumatoid arthritis and normal subjects. Deficient or excessive circulating leukocyte surface IL-10R expression therefore does not seem to play a role in the pathogenesis of SLE or rheumatoid arthritis. Functional IL-10R studies would be of interest.

Introduction

Interleukin-10 (IL-10) is a major regulatory cytokine and has a multitude of immunomodulatory effects on the immune system. It plays a particular role in the development of cells of the monocyte/macrophage and dendritic cell lineages: it augments the growth and differentiation of monocytes into macrophages (1,2) and increases phagocytosis (3). IL-10 also inhibits the maturation of monocytes into dendritic cells (1) and reduces the production of inflammatory cytokines by dendritic cells (4).

The IL-10 receptor is a 90-110 kD protein expressed on a number of leukocyte surface membranes. It belongs to the class II cytokine receptor family, and has been shown to play a role in signal transduction by activating specific STAT proteins and inducing gene transcription (5).

Systemic lupus erythematosus (SLE) is a multisystem autoimmune inflammatory disease of unknown aetiology and a number of IL-10 abnormalities have been associated with SLE (6). A polymorphism in the IL-10 promoter region has been associated with SLE in some populations but not in others (7,8). IL-10 production by monocytes has been shown to be increased in SLE (9). Serum IL-10 is elevated in SLE, and a correlation with disease activity has been reported in some studies (10,11). The actions of IL-10 are mediated through its receptor (IL-10R) but no studies to date have examined the expression of IL-10R in SLE or rheumatoid arthritis. We hypothesised that abnormal IL-10R expression may play a role in SLE disease pathogenesis. We sought to compare the expression of IL-10R on leukocytes in SLE with that on cells from healthy volunteers and patients with rheumatoid arthritis, and correlate this to plasma levels of IL-10, and disease activity and treatment.

Materials and methods

Patients and controls

SLE patients (n=20) and rheumatoid arthritis patients (n=14) fulfilled American College of Rheumatology criteria for diagnosis. Ethical approval was granted and written informed consent obtained from all patients. Normal healthy volunteers (n=14) were recruited from hospital staff.

Clinical information

SLE disease activity was assessed using the SLAM (Systemic Lupus Activity Measure) and the BILAG (British Isles Lupus Assessment Group) scores (12). Rheumatoid arthritis disease activity was assessed using the modified Disease Activity Score (DAS28) (13).

Immunofluorescence labelling

Blood samples were collected in EDTA and immunolabelled within 2 hours. Aliquots of 100 µl whole blood were washed twice in phosphate buffered saline (PB S) to remove any loosely bound IL-10 before immunolabel-
They were then incubated for 15 minutes at 4°C with 10 µl of purified monoclonal mouse anti-human IL-10R (clone 37607.11; R&D systems). 10 µl of PBS was incubated with a control sample to allow subtraction of non-specific staining. The cells were washed once with PBS then incubated with 50 µl of a 1 in 10 dilution of FITC labelled anti-mouse IgG (Sigma) for 15 minutes at 4°C. After a further wash with PBS, red cells were lysed with Immunolyse (Beckman Coulter) according to the manufacturer’s instructions. The cells were washed 3 times with PBS and then suspended in 300 µl of 1% paraformaldehyde in PBS prior to flow cytometric analysis.

Flow cytometry
Flow cytometric analyses were performed on a Coulter EPICS ELITE instrument that was standardized for inter- and intra-run variability by calibration with Immunocheck fluorospheres (Coulter). Monocytes, neutrophils, and lymphocytes were identified by forward and side light scatter properties. Five thousand cells were analysed per sample, and the percentage of cells expressing IL-10R, and their density of surface IL-10R expression, or mean channel fluorescence (MCF), determined in turn after subtraction of non-specific staining as identified by the control histogram (Fig. 1).

Plasma IL-10
Plasma IL-10 levels were determined using a Quantikine human IL-10 ELISA kit (R&D Systems) according to the manufacturer’s instructions. The minimum amount of IL-10 detectable in this assay is 3.9 pg/ml.

Statistical analysis
Results were analysed using Mann-Whitney U tests and Spearman’s correlation coefficients. P values <0.05 were deemed significant.

Results
Patient characteristics
Twenty Caucasian SLE patients (19 female, 1 male, median age 54 years), 14 Caucasian RA patients (all female, median age 54 years) and 14 Caucasian normal healthy volunteers (13 female, 1 male, median age 49.5 years) were studied.

The median number of ACR criteria (for diagnosing SLE) fulfilled was 6 (IQR 5-7). The total number of SLE patients (n = 20) fulfilling each ACR criterion was as follows: malar rash 12, discoid rash 6, photosensitivity 17, oral ulcers 11, arthritis 10, serositis 4, renal disorder 2, neurologic disorder 1, hematologic disorder 16, immunologic disorder 16, and antinuclear antibody 19. The median SLAM score was 7.50 (IQR 6.00 – 9.75), the median total BILAG score was 11.00 (IQR 9.00 – 13.75), and the median SLICC score was 0 (IQR 0 – 1). For the rheumatoid arthritis patients the median DAS28 score was 4.99 (IQR 4.39 – 6.27).

Twelve SLE patients (60%) were receiving corticosteroids at a median dose of 8.75 mg (IQR 7.50 – 10.00 mg). Other medications recorded in the SLE group were hydroxychloroquine (n = 11), NSAIDS (n = 5), azathioprine (n = 1), and methotrexate (n = 1). Four rheumatoid arthritis patients (29%) were receiving corticosteroids at a median dose of 11.00 mg (IQR 10.00 – 14.25 mg). Other medications recorded in the rheumatoid arthritis group were NSAIDS (n = 9), methotrexate (n = 3), d-penicillamine (n = 3), hydroxychloroquine (n = 1), and salazopyrin (n = 1).

IL-10 receptor expression
The median percentage of monocytes expressing IL-10R was 87.8% (IQR 79.1 – 92.6%) in SLE, 87.9% (IQR 77.1 – 91.9%) in rheumatoid arthritis, and 85.8% (IQR 73.8 – 91.0%) in normal controls. The median density of monocyte surface expression of IL-10R (MCF) was 1.62 (IQR 1.31 – 2.20) in SLE, 2.03 (IQR 1.09 – 2.70) in rheumatoid arthritis, and 1.50 (IQR 1.13 – 1.82) in normal controls (Fig. 2). There were no significant differences in monocyte IL-10R expression (either percentage or MCF values) between any of the three groups.

Fig. 1. (a) Flow cytometry scatter plot demonstrating the three leukocyte populations; from left to right are shown lymphocytes, monocytes (circled) and neutrophils. Immunofluorescence profiles: (b) control histogram, (c) FITC-labelled IL-10R staining for gated monocytes (in this sample 89.8% of gated monocytes express IL-10R with an MCF of 2.70).
The median percentage of neutrophils expressing IL-10R was 44.8% (IQR 22.8 – 56.5%) in SLE, 42.2% (IQR 5.7 – 75.7%) in rheumatoid arthritis, and 49.2% (IQR 21.5 – 81.8%) in normal controls. The median density of neutrophil surface expression (MCF) was 0.93 (IQR 0.67–1.14) in SLE, 1.18 (IQR 0.61 – 1.76) in rheumatoid arthritis, and 0.81 (IQR 0.47–0.94) in normal controls (Fig. 3). There were no significant differences in neutrophil IL-10R expression (either percentage or MCF values) between any of the three groups. IL-10R was only minimally expressed on lymphocytes. The mean percentage of lymphocytes expressing IL-10 R was 7.2% (IQR 5.8 – 12.5%) in SLE, 9.8% (IQR 8.3 – 16.0%) in rheumatoid arthritis, and 7.6% (IQR 6.7 – 8.6%) in normal controls. There were no significant differences in lymphocyte IL-10R expression between any of the three groups.

**Plasma IL-10**

IL-10 was detected in the plasma of five SLE patients (25.0%) (range 6.25 – 19.75 pg/ml), four rheumatoid arthritis patients (28.6%) (range 6.75– 24.25 pg/ml), and none of the healthy control subjects. There was no significant difference in plasma IL-10 levels between SLE and rheumatoid arthritis patients.

No significant correlations were observed between leukocyte IL-10R expression and plasma IL-10 levels, or between IL-10R expression and SLAM, BILAG, or DAS28 scores. Prednisolone dose did not correlate with IL-10R expression on any cell type or with plasma IL-10 levels. Plasma IL-10 levels also did not correlate with SLAM, BILAG, or DAS28 scores.

**Discussion**

This is the first reported study to determine the expression of the IL-10 receptor on leukocytes in SLE or rheumatoid arthritis. The strong expression of IL-10R on monocyte surface membranes in all groups is in keeping with IL-10’s role in monocyte/macrophage development and function. We have shown no difference in IL-10R expression on any cell type between any of the three subject groups. This would suggest that deficient or excessive expression of the IL-10 receptor on circulating leukocytes does not seem to be a major factor in the pathogenesis of SLE in this population. However, this does not exclude a functional defect, and molecular and signalling studies are required to substantiate this observation. The increased circulating levels of IL-10 in some SLE patients were confirmed in this study; however, we found no difference in IL-10 levels when SLE patients were compared with rheumatoid arthritis patients. Increased IL-10 levels have previously been described in rheumatoid arthritis (14). It is possible that the effect of IL-10 in rheumatoid arthritis may be counterbalanced by the opposing effects of elevated TNF alpha and other Th 1 type cytokines which are not elevated in SLE. Excess IL-10 in SLE may also impair the clearance of apoptotic cells by altering the expression of clearance-associated molecules, such as CD44, on monocyte/macrophage surface membranes (15).

In summary, although IL-10 may play a central role in SLE pathogenesis, abnormal expression of IL-10R on circulating leukocyte cell surface membranes does not appear to be present in this population of SLE patients. Fur-
ther functional IL-10R studies would be of great interest.

Acknowledgements
The authors thank Mr. G.F. Clarke for his help with flow cytometry and Mrs. E.M. Fleming for her help with the IL-10 ELISA.

References
5. LAI CE, RIPPERGERJ, MORELIA KK et al.: Receptors for interleukin (IL)-10 and IL-6-type cytokines use similar signaling mechanisms for inducing transcription through IL-6 response elements. *J Biol Chem* 1996; 271: 13968-75.
9. ANDERSEN LS, PETERSEN J, SVENSON M, BENDTZEN K: Production of IL-1beta, IL-1 receptor antagonist and IL-10 by mononuclear cells from patients with SLE. *Autoimmun -unity* 1999; 30: 235-42.