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The CD14+ CD16+ monocyte subset in rheumatoid arthritis and systemic lupus erythematosus

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Abstract Most human peripheral blood monocytes strongly express surface CD14, but not CD16 (CD14+/CD16–). A smaller group of monocytes express lower levels of CD14 and also express CD16 (CD14+/CD16+). This subgroup has different functional characteristics and is expanded in a number of disease states. We aimed to determine the percentage of circulating CD14+/CD16+ monocytes in rheumatoid arthritis and systemic lupus erythematosus (SLE) and relate this to disease measures. Peripheral blood was sampled from 31 SLE patients, 19 rheumatoid arthritis patients, and 19 healthy controls. The percentage of CD14+/CD16+ monocytes was determined by immunofluorescence labelling and dual colour flow cytometry. The percentage of CD14+/CD16+ monocytes was significantly lower in rheumatoid arthritis (median 4.90%) than in normalsubjects (median 7.30%, $P = 0.014$), and in rheumatoid arthritis than in SLE patients (median 9.40%, $P = 0.009$). The percentage of CD14+/CD16+ monocytes in SLE was not significantly different from that in healthy subjects. This lower percentage of CD14+/CD16+ monocytes in rheumatoid arthritis may be important in the pathogenesis of this disease.

Keywords CD14 · CD16 · Monocyte · Rheumatoid · Systemic lupus erythematosus

Introduction

Monocyte-derived macrophages and dendritic cells have important roles in the pathogenesis of both rheumatoid arthritis and SLE, two multisystem inflammatory diseases of unknown aetiology. In rheumatoid arthritis, macrophages are prominent in the synovium and are thought to play a central role in the destruction of cartilage and bone by the production of inflammatory cytokines. In SLE, dendritic cells function as antigen-presenting cells, while a defect in the macrophage clearance of apoptotic cells may be important in disease pathogenesis.

Human peripheral blood monocytes can be subdivided on the basis of their expression of CD14 and CD16 into those strongly expressing CD14, but not CD16 (CD14+/CD16–), and those expressing lower amounts of CD14 but also expressing CD16 (CD14+/CD16+) [1]. The CD14+/CD16+ monocytes may have different phagocytosing and antigen-presenting capabilities than CD14+ monocytes [2]. Increased proportions of the CD14+/CD16+ subgroup have been described in a number of disease states including sepsis, tuberculosis, acquired immunodeficiency disease (AIDS), and solid tumours, while a decreased proportion of CD14+/CD16+ cells has been described in ultramarathon runners [1]. No studies to date have examined the relative proportions of these two monocyte subgroups of rheumatic disease.

Materials and methods

Patients and controls

Rheumatoid arthritis patients ($n = 19$) and SLE patients ($n = 31$) fulfilling American College of Rheumatology (ACR) criteria for diagnosis were recruited from rheumatology outpatient clinics and inpatient wards. Patients receiving parenteral corticosteroids, cyclophosphamide, or tumour necrosis factor blocking agents were excluded from the study. Ethics approval was granted and written informed consent obtained from all patients. Normal healthy volunteers ($n = 19$) were recruited from hospital staff.
Clinical information

Rheumatoid arthritis disease activity was assessed using the modified Disease Activity Score (DAS28) [3]. Systemic lupus erythematosus disease activity was assessed using the SLAM (Systemic Lupus Activity Measure) and the BILAG (British Isles Lupus Assessment Group) scores [4]. Total BILAG score was calculated by attributing scores of 4, 3, 2, and 1 to letters a, b, c, and d, respectively, for each group of disease manifestations. Full blood picture, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) by nephelometry, and complement C3 and C4 fractions were measured in all patients. Serum antibodies to dsDNA were measured by the Farr technique in the SLE patients.

Immunofluorescence labelling

Blood samples were collected in EDTA and immunolabelled within 2 h. Aliquots of 100 μl whole blood were incubated for 15 min at 4°C with 10 μl of phycoerythrin (PE)-labelled monoclonal antihuman CD14 (clone TUK4, Dako), and 10 μl of fluorescein isothiocyanate (FITC)-labelled monoclonal antihuman CD16 (clone DJ130c, Dako). FITC- and PE-labelled IgG (Dako) was incubated with a control sample to allow subtraction of nonspecific staining. The cells were washed three times with phosphate-buffered serum (PBS); then red cells were lysed with Immunolyse (Beckman Coulter) according to the manufacturer’s instructions. The cells were washed once with phosphate-buffered serum (PBS); then red cells were lysed with Immunolyse (Beckman Coulter) according to the manufacturer’s instructions. The cells were washed once 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 intrarun variability by calibration with Immunochek fluorospheres (Coulter). Monocytes were identified by forward and side light scatter properties. Five thousand cells were analysed per sample, and the percentage of CD14+/CD16– and CD14+/CD16+ monocytes determined in turn after subtraction of nonspecific staining as identified by the isotype control histogram (Fig. 1).

Statistical analysis

Mann Whitney U tests were performed to analyse the significance of differences between the groups. Spearman’s correlation coefficients were calculated to study the relations of monocyte groups with measures of disease activity and treatment. P values of <0.05 were deemed significant.

Results

Patient characteristics

Thirty-one Caucasian SLE patients (28 female, three male, median age 50 years), 19 Caucasian RA patients (17 female, two male, median age 51 years), and 19 Caucasian normal healthy volunteers (17 female, two male, median age 48 years) were studied. Median DAS28 score was 4.73 (interquartile range 4.24–6.14), median SLAM score 8 (interquartile range 6–11), and median BILAG score 11 (interquartile range 9–15).

Monocyte subsets

The proportion of CD14+/CD16+ monocytes was lower in rheumatoid arthritis (median 4.9%, interquartile range 2.6–6.6%) than in normal controls (median 7.3%, interquartile range 5.5–9.9%, P = 0.014) (Fig. 2). The proportion of CD14+/CD16+ monocytes was also lower in rheumatoid arthritis patients than in SLE (median 9.4%, interquartile range 5.8–11.5%, P = 0.009). There was no significant difference between the percentage of CD14+/CD16+ monocytes in SLE and healthy normal subjects.

Disease activity and treatment

No correlations were seen between the proportion of CD14+/CD16+ monocytes and DAS28, SLAM, or BILAG scores. Seven rheumatoid arthritis patients (37%) were receiving oral corticosteroids. Of these, the median dose was 10 mg (interquartile range 5.0–12.0). Other drugs recorded were nonsteroidal anti-inflammatory drugs (NSAIDs) (n = 11), methotrexate (n = 3), sulphasalazine (n = 1), hydroxychloroquine (n = 1), leflunomide (n = 1), and d-penicillamine (n = 4). Nineteen SLE patients (61%) were receiving oral corticosteroids. Of these, the median dose was 10 mg (interquartile range 7.5–15.0). Other drugs recorded were hydroxychloroquine (n = 18), NSAIDs (n = 10), azathioprine (n = 6), and methotrexate (n = 2). There was no significant difference in the proportion of CD14+/CD16+ monocytes between those patients receiving corticosteroids and those not, in either rheumatoid arthritis or SLE. Also, the dose of corticosteroids did not correlate with the proportion of CD14+/CD16+ monocytes in rheumatoid arthritis or SLE.

When all patients receiving corticosteroids were excluded, there was still a significantly lower proportion of CD14+/CD16+ monocytes in rheumatoid arthritis patients (median 3.6%, interquartile range 2.52–7.67, n = 12) than in normal subjects (median 7.3%, interquartile range 5.5–9.9%, P = 0.025, n = 19) and in rheumatoid arthritis compared with SLE (median 8.0%, interquartile range 6.17–10.85, P = 0.039, n = 12). There was still no significant difference in the proportion of CD14+/CD16+ monocytes between SLE patients and normal subjects. There was no difference in the proportion of CD14+/CD16+ monocytes between groups taking any other drugs in rheumatoid arthritis or SLE.

Discussion

This is the first reported study of CD14+/CD16+ and CD14++/CD16– monocyte subgroups in rheumatoid arthritis and SLE. We have shown that patients with rheumatoid arthritis have a lower proportion of circulating CD14+/CD16+ monocytes than either normal healthy controls or patients with SLE. No correlations were seen with measures of disease activity, suggesting that this may be a feature of the disease itself rather than simply related to active inflammation. No correlations