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ORIGINAL ARTICLE

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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 normal subjects (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

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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 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 three 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 intrarun variability by calibration with Immunocheck 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

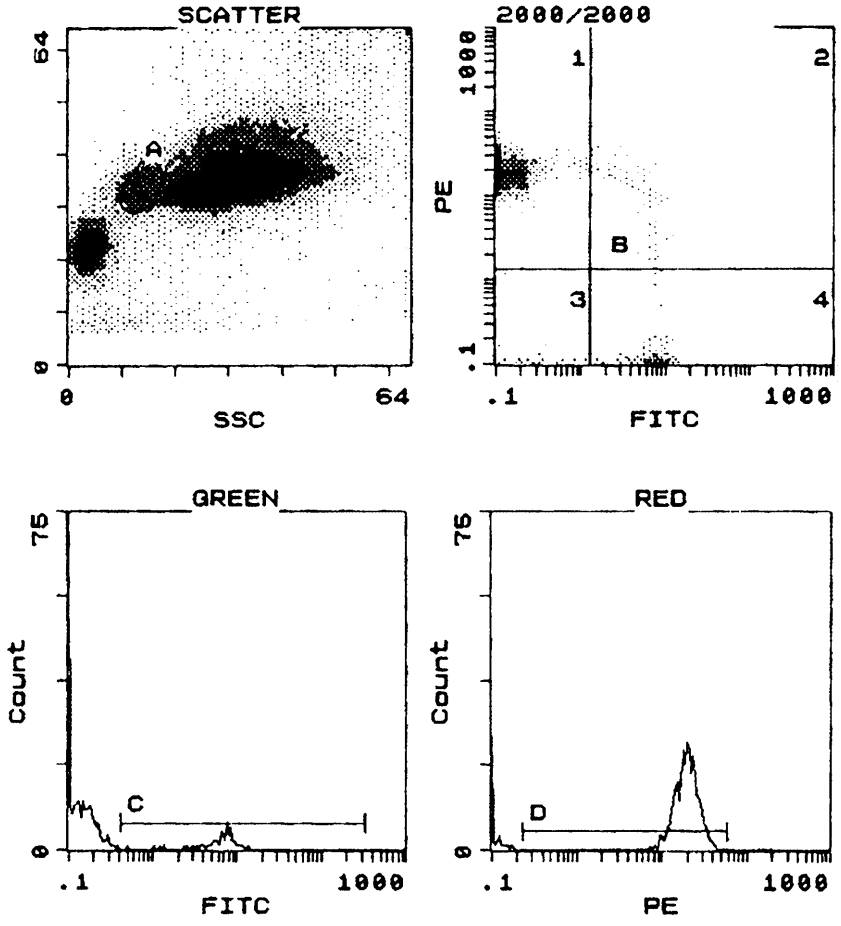

Fig. 1a–d Flow cytometry scatter plots demonstrating **a** the three leukocyte populations – from *left* to *right* lymphocytes, monocytes (*circled*), and neutrophils, **b** Fluorescein isothiocyanate (FITC)-labelled CD16 and phycoerythrin (PE)-labelled CD14 staining of the gated monocyte population – CD14++/CD16- monocytes in box 1, CD14+/CD16+ monocytes in box 2 (5.4% of monocytes in this sample), **c** a FITC-labelled CD16 histogram, and **d** a PE-labelled CD14 histogram

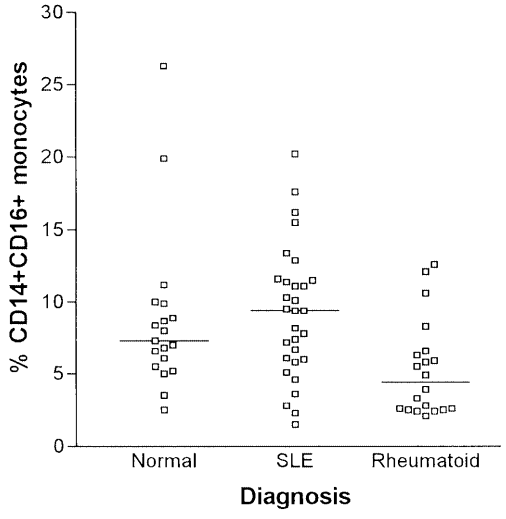

Fig. 2 Scatter plot demonstrating the percentage of CD14+/CD16+ monocytes in normal, rheumatoid arthritic, and SLE subjects. Each point represents one patient, and the median value for each is shown

with drug treatment were seen. Specifically, those patients taking corticosteroids did not have different monocyte subgroup proportions, and the dose of corticosteroids did not correlate with monocyte subgroup proportions. The proportion of CD14+/CD16+ monocytes has been shown to drop following corticosteroid treatment in multiple sclerosis [5], and our study is therefore of interest in comparing two different disease groups receiving corticosteroids, one (rheumatoid arthritis) with a lower proportion of CD14+/CD16+ monocytes and another (SLE) with a proportion of CD14+/CD16+ monocytes similar to that in normal subjects. This suggests that the diseases themselves are associated with different proportions of the monocyte subgroups.

The CD14 and CD16 molecules are functionally important receptors. CD14 is the major receptor for lipopolysaccharide (LPS) and also has a role in the recognition and phagocytosis of apoptotic cells [6, 7]. CD16 is the Fcγ receptor type III. CD16 expression increases with culture of monocyte/macrophages, and it has a major role in mediating immunophagocytosis [8]. The CD14++/CD16– group is numerically the dominant group, accounting for over 90% of circulating monocytes in most studies of normal subjects. Phenotypically, the CD14+/CD16+ monocytes have typical monocyte morphology with irregular nuclei and are smaller than CD14++/CD16– monocytes [2].

The capacity for phagocytosis and reactive oxygen production may be lower in CD14+/CD16+ monocytes [2], and these cells may be more potent antigen-

presenting cells, as they express higher levels of major histocompatibility complex (MHC) class II molecules [9]. They also secrete different amounts of cytokines such as interleukin 10 (IL-10) compared with CD14++ monocytes [10]. Some studies have suggested that CD14+/CD16+ monocytes are more mature than CD14+ monocytes, with features in keeping with tissue macrophages [9]. Another group has demonstrated that CD14+/CD16+ cells differentiated in vitro from peripheral blood monocytes exhibit dendritic cell characteristics [11].

Why should the proportion of monocyte subsets differ in the groups we studied? One explanation may involve the different levels of circulating cytokines in these conditions. Rheumatoid arthritis has been described as a predominantly Th1-type disease, with elevated levels of cytokines such as tumour necrosis factor alpha (TNF alpha), interleukin (IL)-1, and IL-2, while SLE is a Th2 type disease with elevated levels of IL-10 [12]. Interleukin-10 has been shown to induce CD16 in blood monocytes in culture [13]. We measured plasma IL-10 levels by enzyme-linked immunosorbent assay (ELISA) and found no correlation with the proportion of CD14+/CD16+ monocytes (data not shown). It would also be of interest to determine the relationships of CD14+/CD16+ monocytes with other cytokines such as TNF alpha, IL-1, and IL-2. Macrophage colony-stimulating factor (M-CSF) has also been shown to upregulate CD16 expression in cancer patients [14], while granulocyte-macrophage colony-stimulating factor (GM-CSF) downregulated CD16 expression [15]. The relative proportions of various other cytokines in vivo may have a role in determining the expression of CD14 and CD16, but this is an area which requires clarification by further study.

It is difficult at present to attribute a pathophysiological role to the altered monocyte subgroup proportions in each disease, given our incomplete understanding of their nature. It is possible that the lower percentage of CD16+ on monocytes (and therefore higher percentage of CD14++ monocytes) in rheumatoid arthritis may contribute to increased adhesion, phagocytosis, and cytokine secretion. It would be of interest to determine the expression of CD14 and CD16 in synovial membrane and synovial fluid monocytes in rheumatoid arthritis, as this is where much of the disease-associated damage occurs in rheumatoid arthritis.

This is however an interesting area for further studies, as potential treatments for rheumatoid arthritis or SLE may involve modifying the relative monocyte subgroup proportions in each condition.

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