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Altered chemokine receptor expression in the peripheral blood lymphocytes in polymyositis and dermatomyositis

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ABSTRACT

Objective: To examine the expression of chemokine receptors in different peripheral blood T-cell subsets in patients with polymyositis (PM) and dermatomyositis (DM).

Methods: We used flow cytometry to measure the frequencies of chemokine receptors CXCR3 and CCR4 expression in the $CD4^+$ or $CD8^+$ lymphocytes. Enzyme linked immunosorbent assays were also used to measure the concentrations of C-X-C motif chemokine 10 (CXCL10), thymus and activation regulated chemokine (TARC) and macrophage derived chemokine (MDC).

Results: Comparing to 20 healthy controls, $\%CD4^+CXCR3^+$ and $\%CD8^+CXCR3^+$ T cells significantly decreased in 33 DM patients, and $\%CD8^+CXCR3^+$ cells decreased in 24 PM patients, but $\%CD4^+CCR4^+$ and $\%CD8^+CCR4^+$ cells did not significantly change in both the PM and DM patients. Accordingly, the Th1/Th2 polarization, analyzed as the balance obtained after dividing $\%CD4^+CXCR3^+$ cells by $\%CD4^+CCR4^+$ cells, showed a significant reduction in DM. The serum concentration of $CXCR3^+$ ligand, CXCL10, significantly increased and negatively correlated with circulating $\%CD4^+CXCR3^+$ cells in DM patients. There was no significant change of TARC and MDC in PM and DM patients. Furthermore, $\%CD4^+CXCR3^+$ cells decreased more severely in the patients with interstitial lung disease.

Conclusions: The present results indicate that the distributions of circulating $CXCR3^+$ T-cells differ among the PM and DM cases. Our findings suggest a pathogenic difference between PM and DM.

1. Introduction

Polymyositis (PM) and dermatomyositis (DM) are autoimmune myositis of unknown origin, characterized by marked proximal and symmetric muscle weakness [1,2]. Muscle biopsy in PM and DM patients commonly observed that T lymphocytes and macrophages [3] infiltrate into the muscle fibers. Despite the similarity, there are some marked differences between them. In PM, the perivascular infiltration in muscle biopsies is composed predominantly of $CD8^+$ T cells and macrophages [4,5], whereas inflammatory infiltration in DM mainly consist of $CD4^+$ T cells as well as B cells and macrophages [6,7]. $CD4^+$ T cells are also the predominant component of infiltrating cells in the skin lesions of DM, and both Th1 and Th2 cells were found in the infiltration [8]. Thus, different subsets of T cells may be involved in the pathogenesis of PM and DM.

Chemokine and chemokine receptor play critical roles in the migration and transformation of T cell [9]. Interaction between chemokine and chemokine receptor may participate in the pathogenesis of PM and DM by mediating the T cell infiltration [10]. C-X-C motif chemokine 10 (CXCL10) is a member of chemokines specially attracts activated T cell expressing the chemokine (C-X-C motif) receptor 3 (CXCR3). CXCL10/CXCR3 contributes to the recruitment and activation of Th1 cells [11]. Abundant expression of CXCL10/CXCR3 has been found on the T cells of DM patients [8,12,13], suggesting that the CXCL10/CXCR3 interaction, reflecting Th1-mediated immunity, is important in the immunopathogenesis of DM.

Thymus and activation regulated chemokine (TARC) and macrophage derived chemokine (MDC) are ligands for a chemokine (CC motif) receptor (CCR)4, which is preferentially expressed on Th2 cells [11,14]. CCR4 was found to express in the blood vessels and regenerating muscle fibers in the samples of PM and DM patients [15]. Thus, the Th2-mediated immunity is also involved in the pathogenesis

Abbreviations: PM, polymyositis; DM, dermatomyositis; IP-10, IFN-γ-inducible protein 10; TARC, thymus and activation regulated chemokine; MDC, macrophage derived chemokine; IIM, idiopathic inflammatory myopathies; IL, interleukin; IFN, interferon; Th, T helper; CADM, clinically amyopathic dermatomyositis; ILD, interstitial lung disease; PBMC, peripheral blood mononuclear cells; FACS, fluorescence activated cell sorter

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of idiopathic inflammatory myopathies.

Though biopsy is a good method for the pathological study of myopathies, it is difficult to perform a large sample statistical analysis because of the limited availability of specimens derived from pathognomonic lesions. Therefore, studies on peripheral blood samples are necessary to characterize the clinic features of DM/PM and gain better insight into the pathogenesis. For this reason, we used a flow cytometric assay to examine the expression of chemokine receptors in peripheral blood $CD4^+$ and $CD8^+$ T cells of PM and DM patients. We also measured the levels of chemokines in the sera and analyzed whether they are correlated with the expression of chemokine receptors in circulating T cells.

2. Methods

2.1. Patients and controls

In this prospective study, we collected the newly diagnosed PM or DM (including clinically amyopathic dermatomyositis, CADM) patients between 2015 and 2016 from the Department of Rheumatology and Immunology of the First Affiliated Hospital of China Medical University. The criteria of Bohan and Peter [16] and following the modified Sontheimer's definition [17] were used as the diagnosis criterion. We only selected the patients that had a disease duration shorter than one month and had not received immunosuppressive treatment. Exclusion criteria included presence of overlap syndrome, infection and biological therapy. The local ethics committee approved all the experiment protocols. Informed consents were obtained from the subjects.

2.2. Clinical and laboratory data

Interstitial lung disease (ILD) was diagnosed according to the findings of high-resolution computed tomography (HRCT) [18]. The results of routine blood biochemical examination, such as alanine aminotransferase (ALT), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and lactate dehydrogenase (LDH) levels, were measured at the time of enrollment and recorded for further analysis. Additional peripheral blood samples were divided into two parts, one was centrifuged shortly after clot formation to obtain serum, and the other was anticoagulated to obtain peripheral blood mononuclear cells (PBMC). The serum samples were stored at −80 °C until tested.

2.3. Flow cytometry

PBMCs were isolated by means of lymphocyte separation medium (Ficoll-Paque™ PLUS, Amersham Biosciences) and density gradient centrifugation. The cell surface of PBMCs was stained with fluorochrome-conjugated mAbs by a 30 min incubation at 4 °C. The following antibodies were used for surface staining, FITC-conjugated antibody: CD4 (clone RPA-T4; BD Biosciences); PE-Cy7-conjugated antibody: CD8 (clone RPA-T8; BD Biosciences); APC-conjugated antibody: CXCR3 (clone 1C6/CXCR3; BD Biosciences); PE -conjugated antibody: CCR4 (clone 1G1; BD Biosciences) and their Isotype controls. Stained samples were acquired by using FACSCan (BD Biosciences). The results of flow cytometry were acquired with CellQuest software and analyzed in FlowJo v10 software (Tree Star, Ashland, OR, USA). The cells were analyzed after gating on $CD4^+$ or $CD8^+$ lymphocytes, and the expression of CCR4 and CXCR3 was shown on the y-axis (Fig. 1). The cut point to consider the expression of CXCR3 and CCR4 as high was obtained after determining the maximum expression in a group of healthy donors (± 0.25).

2.4. Measurement of serum chemokine concentrations by ELISA

Serum concentrations of CXCL10, TARC, and MDC were measured by ELISA according to the manufacturer's protocol (R & D Systems, Minneapolis, MN, USA).

2.5. Statistical analysis

Data are presented as mean ± standard deviation (SD). One-way ANOVA and multiple comparison (Tukey's test) were used to examine the differences between the healthy controls and the patients with PM or DM. Pearson regression was used to assess correlations between variables. All the statistical analyses were conducted using SPSS 17.0 software. A significant difference was defined as two-tailed p-values less than 0.05.

3. Results

3.1. Clinical characteristics of PM and DM patients

Fifty-seven patients suffering from PM (n = 24) or DM (n = 33, including 16 CADM) and 20 matched healthy controls were enrolled in this study. The clinical profile was characterized as Table 1. From our data, 66.7% DM patients and 62.5% PM patients had ILD at presentation. PM and DM patients had a lymphocytopenia, significant decrease in lymphocyte count comparing to healthy controls ($p < 0.05$, ANOVA and Tukey's test). There was an abnormality of ESR, CRP and ALB, et al. in PM and DM patients.

3.2. Expression of CXCR3 and CCR4 on lymphocytes derived from PM/DM patients

The expression of CXCR3 and CCR4 on the cell surface of lymphocytes was examined by flow cytometry. The frequency of $CXCR3^+$ and $CCR4^+$ was counted in the $CD4^+$ and $CD8^+$ T cells, respectively. Fig. 1 shows a representative example of fluorescence activated cell sorter (FACS) dot-plots for CXCR3–CD4, CXCR3–CD8, CCR4–CD4, and CCR4–CD8. Statistical analysis revealed a significant reduction of the percentage of $CD4^+CXCR3^+$ (%$CD4^+CXCR3^+$) cells in DM patients, comparing to healthy controls and PM patients (ANOVA followed Tukey's test, $p < 0.05$, Fig. 2A). However, there was no significant difference of %$CD4^+CXCR3^+$ between PM patients and healthy controls. Both DM and PM patients showed a decreased %$CD8^+CXCR3^+$ (Fig. 2B), which was lower in DM patients than that in PM patients. For CCR4, we did not find a significant difference of %$CD4^+CCR4^+$ or %$CD8^+CCR4^+$ between the healthy controls and DM/PM patients (Fig. 2C and D).

Because $CD4^+CXCR3^+$ and $CD4^+CCR4^+$ cells can be considered as a marker of Th1 and Th2 responses, respectively, and a bias of Th1/Th2 is a characteristic of many immunological disorders [19,20], we calculated the ratio of $CD4^+CXCR3^+/CD4^+CCR4^+$ in the healthy controls and DM/PM patients. As a result, DM patients showed a significantly lower $CXCR3^+/CCR4^+$ than PM patients and healthy controls (ANOVA followed Tukey's test, $p < 0.05$, Fig. 2E). No significant difference was detected between PM patients and healthy controls.

3.3. Serum levels of CXCR3 and CCR4 ligands in healthy controls and DM/PM patients

We further examined the serum concentrations of CXCL10, TARC and MDC, which are ligands of CXCR3 and CCR4, respectively. Both PM and DM patients showed a significantly higher level of CXCL10 comparing to healthy controls, and the level of CXCL10 in DM was further higher than that in PM (ANOVA followed Tukey's test, $p < 0.05$, Fig. 3A). On the other hand, there was no significant difference in the levels of TARC and MDC between the healthy controls and PM/DM patients (Fig. 3B and C).

Furthermore, the concentration of CXCL10 was negatively correlated with % $CXCR3^+CD4^+$ cells and $CXCR3^+CD8^+$ cells (Fig. 4A and B) in DM patients. There was no significant correlation