Rheumatoid arthritis (RA) is a systemic autoimmune disease which is characterized by chronic, progressive, and invasive arthritis as the main manifestation. It develops gradually and eventually leads to joint deformity and loss of function without formal treatment and has a high disability rate. RA is distributed all over the world, the incidence rates in different populations range from 0.01% to 0.05%, and the prevalence rates range from 0.18% to 1.07%. The incidence rates have certain racial differences and rank from Indians to white Caucasians to yellow Asians. At present, the etiology and pathogenesis of RA is not clear, but it is determined that the pathogenesis of RA is closely related to immune dysfunction.

The adhesion molecule CD62P plays an important role in the pathogenesis of autoimmune diseases, tumors, atherosclerosis, asthma, and other diseases. CD154 (CD40 ligand, CD40L) belongs to the tumor

Expression of CD62P and CD154 in peripheral blood of patients with rheumatoid arthritis and their correlation with clinical indexes

Shiping Qu, Chunyi Yu, Qian Xing, Haisheng Hu and Haiyan Jin

Abstract
The aim of this study is to investigate the expression of CD62P and CD154 in peripheral blood of patients with rheumatoid arthritis (RA) and their correlation with the clinical indexes of RA. A total of 60 RA patients diagnosed and treated in the Department of Rheumatism in our hospital from January to December 2016 were selected as the RA group, and 60 cases of healthy subjects were selected as the control group. CD62P and CD154 levels in peripheral blood were determined by flow cytometry using the FACS Vantage flow cytometer, and the correlation analysis with the clinical indexes of RA patients were conducted. The levels of CD62P and CD154 in the peripheral blood of RA group were 28.75% ± 1.48% and 26.84% ± 1.03%, respectively, which were significantly higher than those of the control group (P < 0.05). The levels of white blood cell (WBC), platelet (PLT), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), C-reactive protein (CRP), and interleukin (IL)-37 in the RA group were significantly higher than those in the control group (P < 0.05). Pearson test showed that CD62P and CD154 levels in the peripheral blood in the RA group were positively correlated with serum WBC, PLT, ESR, RF, CRP, IL-37, and disease activity score 28 (DAS28) (P < 0.05), but not correlated with disease course (P > 0.05). The expression of CD62P and CD154 in peripheral blood of patients with RA was upregulated, and their expression levels were correlated with the activity of RA and the degree of joint lesion.

Keywords
CD154, CD62P, peripheral blood, rheumatoid arthritis

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The adhesion molecule CD62P plays an important role in the pathogenesis of autoimmune diseases, tumors, atherosclerosis, asthma, and other diseases. CD154 (CD40 ligand, CD40L) belongs to the tumor
necrosis factor superfamily and is mainly expressed on the surface of CD4+ T lymphocytes. CD154 can combine with its receptor CD40 (expressed on the surface of B lymphocytes) and mediate the activation and proliferation of B cells, which will help promote production of humoral immunity. However, there are few reports about CD62P and CD154 in patients with RA. In this study, by detecting the expression of CD62P and CD154 in peripheral blood of patients with RA, we analyzed their correlation with the activity degree of RA. We also investigated their role in the pathogenesis and progression of RA and provided experimental basis for further study of pathogenesis characteristics of patients with RA.

Materials and methods

General data

A total of 60 RA patients diagnosed and treated in the Department of Rheumatism in our hospital from January to December 2016 were selected as the RA group. The general data of these patients were as follows: 20 males and 40 females; aged from 19 to 70 years old with a mean age of 56.4 ± 2.5 years; and the disease activity score 28 (DAS28) for disease activity was 3–5 with a mean score of 4.5 ± 0.7, all patients were Han nationality. All RA patients met the criteria for RA revised by the American College of Rheumatology in 1987, except for pregnant or lactating women and other rheumatic diseases. A total of 60 healthy people in our hospital were selected as control group, including 20 males and 40 females, aged 20–69 years with a mean age of 56.2 ± 2.3 years. All of them were Han nationality. There were no significant differences in age and sex between the RA group and the control group at baseline (P > 0.05). All subjects were informed and signed consent to participate in the study.

Reagents and instruments

FITC-labeled anti-CD62P and anti-CD154 monoclonal antibodies, sheep anti-mouse FITC IgG, and PE IgG negative controls were all purchased from Immunotech, France. Enzyme-linked immunosorbent assay (ELISA) kits for detection of C-reactive protein (CRP) and interleukin (IL)-37 were purchased from Jiangsu Green Leaf Biotechnology Co., Ltd, China. High-speed refrigerated centrifuge was purchased from Sichuan Instrument Co. Ltd. Refrigerator was purchased from Haier Group Company, China. Automatic blood cell analyzer was purchased from Ji’nan Lai Bao Medical Devices Company, China.

Sample collection and processing

Three milliliters of blood from the cubital vein was obtained in the morning from all subjects and injected into the EDTA anticoagulant tube and gently mixed. In order to reduce man-made and in vitro factors affecting platelet activation, this step was charged by specific person and samples were processed as soon as possible within 45 min. Samples were centrifuged at 336g for 8 min at 4°C, and the supernatants were obtained as platelet-rich plasma (PRP).

Add 10 μL of PRP and FITC/PE-labeled monoclonal antibody into one tube, and 10 μL of PRP and FITC/PE-labeled IgG isotype into another tube. Add 30 μL of phosphate-buffered saline (PBS) dilution into the two tubes and mix. The tubes are kept at room temperature in the dark for 20 min and fixed with 1% paraformaldehyde PBS liquid and then placed at 4°C for detection.

Flow cytometry

Double color labeling method and 488 nm He-Ne luminescence as the light source were used. The front angle and lateral angle scattered light double parameter scatter plots were used to set the gate of the platelet group, and the isotype control group was set as the negative group. The coefficient of variation was adjusted to <2%, and 10,000 platelets were collected for detection. All light scattering and fluorescence data were saved. After testing, data were processed and analyzed by the software ELITE4.5. Results were expressed as the positive percentage of CD62P and CD154 expressed on platelet surface.

Detection of other laboratory indexes

Peripheral white blood cell (WBC) count and platelet (PLT) count were detected by all automatic blood cell analyzer. Erythrocyte sedimentation rate (ESR) was detected by the Westergren method. Rheumatoid factor (RF) was detected by latex agglutination assay. CRP and IL-37 were detected by ELISA.
Statistical analysis

SPSS 21 software was used for statistical analysis. Data are expressed as the mean ± SD. After examining the normality and homogeneity of variance of laboratory indexes of each group, comparisons between groups were conducted using t test. If data were not in accordance with normal distribution and homogeneity of variance, data were analyzed after the log conversion or square root anti sine conversion. Pearson test was used for correlation analysis. $P < 0.05$ means statistically significant.

Results

Comparison of CD62P and CD154 levels in peripheral blood between two groups

The levels of CD62P and CD154 in the peripheral blood of the RA group were significantly higher than those of the control group ($P < 0.05$), as shown in Figure 1.

Comparison of laboratory indexes between the two groups

The levels of WBC, PLT, ESR, RF, CRP, and IL-37 in the RA group were significantly higher than those in the control group ($P < 0.05$), as shown in Table 1.

Correlation analysis

Pearson test showed that CD62P and CD154 levels in the peripheral blood in the RA group were positively correlated with serum WBC, PLT, ESR, RF, CRP, IL-37, and DAS28 ($P < 0.05$), but not correlated with disease course ($P > 0.05$), as shown in Table 2 and Figure 2.

Discussion

RA is an autoimmune disease characterized by widespread systemic inflammation mainly involving peripheral joints, it is mainly manifested as symmetric and chronic inflammatory disease with multiple joints. It is clinically manifested as morning stiffness, symmetric multi-joints involvement, joints pain, or deformity, resulting in movement dysfunction, and is one of the most common rheumatic diseases. The pathogenesis of RA is still unclear based on current research, and studies have confirmed that RA is caused by many factors, such as environment, heredity, viruses, bacteria, sex hormones, and neuropsychiatric state.

CD62P is one of the integrin family members of the adhesion molecules. CD62P is released from the platelet surface into the blood and becomes soluble CD62P, which may mediate the rolling of WBCs such as lymphocytes, monocytes, and neutrophils onto endothelial cells and promote the adhesion and
migration in synovial tissue, and it plays an important role in the initiation and progression of the pathology of RA. Hosaka et al. found that the level of CD62P in synovial fluids of patients with RA was higher than that in patients with osteoarthritis (OA) and other arthritis patients. CD62P is the main mediator of adhesion between monocytes and endothelial cells during RA synovitis. Anti-CD62P antibodies

Table 2. Correlation between CD62P and CD154 in peripheral blood and related indexes in RA group.

|      | WBC    | PLT    | ESR    | RF     | CRP    | IL-37   | Course | DAS28 Score |
|------|--------|--------|--------|--------|--------|---------|--------|-------------|
| **r** | 0.512  | 0.505  | 0.592  | 0.637  | 0.596  | 0.675   | 0.126  | 0.475       |
| **P** | <0.05  | <0.05  | <0.05  | <0.05  | <0.05  | <0.05   | >0.05  | <0.05       |

Table 2. Correlation between CD62P and CD154 in peripheral blood and related indexes in RA group.

|      | WBC    | PLT    | ESR    | RF     | CRP    | IL-37   | Course | DAS28 Score |
|------|--------|--------|--------|--------|--------|---------|--------|-------------|
| **r** | 0.543  | 0.582  | 0.611  | 0.691  | 0.604  | 0.668   | 0.134  | 0.483       |
| **P** | <0.05  | <0.05  | <0.05  | <0.05  | <0.05  | <0.05   | <0.05  | <0.05       |

WBC: white blood cell; PLT: platelet; ESR: erythrocyte sedimentation rate; RF: rheumatoid factor; CRP: C-reactive protein; IL: interleukin; RA: rheumatoid arthritis; DAS28: disease activity score 28.

Figure 2. Scatter plots of correlation between CD62P and CD154 in peripheral blood and related indexes in RA group.
can inhibit the adherence of monocytes from RA peripheral blood to synovial microvascular endothelial cells. The high expression of CD62P in platelets of peripheral blood can directly mediate the adhesion of platelets to vascular endothelial cells and transfer to the synovial membrane of joints to play a role. Ertenli et al. showed that soluble CD62P levels in RA patients with increased platelets were higher than those in RA patients with normal platelets, and their levels were positively correlated with joint activity index and platelet count. Results of this study are consistent with the results of Ertenli et al. and other studies, indicating a significant platelet activation in the pathogenesis of RA.

CD154 is expressed on the surface of lymphocytes and platelets. There is no CD154 on the surface of platelets in resting state, and sCD154 can occur transiently when platelets are activated. sCD154 can upregulate the expression of CD62P on platelet surface, and 90% of CD154 in circulating blood comes from activated platelets. Platelets can combine with CD40 expressed on T cells, B cells, and mononuclear cells and can stimulate the release of cytokines, such as monocyte chemotactic protein 1. It can also regulate the expression and secretion of active factors in T cells and promote inflammation responses and promote B cells to produce IgG type RF. The expression of CD154 in peripheral blood of RA patients increased significantly and was positively correlated with the expression of IgM and IgG RF. CD154 on the surface of activated platelets can combine with CD40 of endothelial cells, macrophages, and smooth muscle cells, which in turn can aggravate inflammatory responses and make the plaque more unstable or delay healing. Persistent plaque instability will lead to more platelet activation. The results of this study showed that the expression of CD154 was significantly higher in peripheral blood of RA patients than that of healthy people, which indicates a significant correlation between CD154 and RA, suggesting that CD154 may be used as a marker of detection index for the diagnosis of RA.

DAS28, CRP, IL-37, and ESR have been widely used in the clinic and in research as indexes to evaluate the activity of RA. The higher the DAS28, the higher the disease activity. In this study, we found that CD62P and CD154 were positively correlated with serum WBC, PLT, ESR, RF, CRP, IL-37, and DAS28 (P < 0.05), but not correlated with the course of disease (P > 0.05). The limitation of this study is that the sample size was small and we only enrolled the Han nationality, and it needs further studies to determine whether the results of this study are related to racial differences.

In conclusion, the expression of CD62P and CD154 in peripheral blood of patients with RA was upregulated, and their expression levels were correlated with the activity of RA and the degree of joint lesion.

Declaration of conflicting interests
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