A Flowcytometry Study of CD55 and CD59 Expression on Erythrocytes in Rheumatoid Arthritis Patients

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ABSTRACT

Background: Inappropriate activation or blockage of the inhibition of complement system could cause tissue damages in autoimmune diseases particularly rheumatoid arthritis (RA). Defect in complement component regulation may cause damages to tissues, on the other hand, or the damaged tissue might affect the unnecessary activation of complement components. **Objective:** To investigate the expression of CD55 and CD 59 complement regulatory proteins in RA patients. **Subjects and Methods:** Fifty proved rheumatoid arthritis patients participated in this study and their blood were collected for investigations. CD55 and CD59 molecules expression on the erythrocytes was assayed using primary monoclonal antibody and secondary FITC conjugated Ab, then the prepared samples were run with a FACSCalibur flowcytometer (Becton-Dickinson) and the obtained data was analyzed using a Cell Quest software package. To evaluate the complement function, CH50 was performed using patient sera. All experiments were done with a matched healthy volunteer group. **Results**: The mean fluorescence intensity for CD55 was 27.6 ± 13.4 arbitrary unit for patients and 68.5 ± 10.5 for healthy group. CD59 mean fluorescence intensity was 314 ± 83 in patient group and 508 ± 56 in healthy volunteers. In addition, there was a significant difference between CH50 in patients (54.5 ± 15.5) and in healthy group (110 ± 20). A significant correlation between CD55 and CD59 expansion on the patient erythrocytes was found ($P = 0.00$, $r = 0.576$). No association was found between CD59, or CD55 with CH50 ($P > 0.05$). **Conclusion:** The expression of CD55 and CD59 is down-regulated on erythrocytes of patients with RA. Change in expression of regulatory complement components in RA may be a useful key for the assessment of disease progression or in patients' follow-up.

Keywords: Autoimmunity, CD55, CD59, CH50, Flowcytometry, Rheumatoid Arthritis

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INTRODUCTION

The complement system is considered to be an important system in the control of infectious agents, with the ability to act as an effector molecule to lyse many microorganisms and cells. In autoimmune diseases like RA, the complement system may become activated under inappropriate circumstances with different pathways and cause self-tissue damages. Several proteins and receptors on self tissue control and regulate the over activation of complement components, some of these include CR1/CD35, decay-accelerating factor (DAF)/CD55, membrane cofactor protein (mcp)/CD46, Protectin/CD59, C8 binding protein, Factor I, Factor H and C1 inhibitor (1,2). It has been reported that the production and the expression of some of the regulatory proteins are altered in auto-immune diseases (3). Jones et al. indicated alteration of surface markers on neutrophils from RA patients (4). In addition, Ross et al. showed loss of CR1 on erythrocytes in patients with systemic lupus erythematosus and other diseases involving auto-antibodies (5). Kumar et al. indicated a lowered expression of C3b receptor on erythrocytes of RA patients (6). A spectrum of changes in complement regulatory proteins expression and activation of complement cascade on erythrocytes of RA is also reported (7). By using flowcytometer, Hatanaka et al. studied the membrane factors including DAF and CD59 in complement-mediated lysis of paroxysmal nocturnal hemoglobinuria erythrocytes (8). However, a reduction in erythrocyte complement receptor 1 (CR1, CD35) and decay accelerating factor (DAF, CD55) in a physiological situation like normal pregnancy has also been indicated (9).

CD55 is a 70-kD glycoprotein that is expressed on peripheral blood, vascular endothelial and extravascular epithelial cell surfaces. It inhibits the activity of C3 convertase of the classical and alternative pathways. It has been reported that patients with paroxysmal nocturnal heamoglobinuria, who lack CD55, accumulate C3b in vivo, suggesting a physiologic role for CD55 (2,10,11).

CD59 is a 18-kD phosphatidylinositol-linked membrane protein that is expressed on erythrocytes, lymphocytes, monocytes, neutrophils, platelets, endothelial/epithelial cells, etc. It blocks C9 binding to C5b-8, preventing membrane attack complex formation and lysis (2,12-14). The present study was conducted to understand in part the role of CD55 and CD59 in the pathogenesis of RA. The study investigates the expression of CD55 and CD59 on erythrocytes and correlates the protein expression with the in vitro complement activity by determining the assessment of CH50 activity in sera of normal controls and RA patients.

PATIENTS AND METHODS

A group of approved RA patients including 10 males and 40 females, mean age 46.8 years, ranges 17-80 years were selected. A healthy volunteer group with no history of autoimmune diseases including RA was involved in the present study. This group consisted of 14 females and 6 males (mean age = 36.4 yr, range = 20-50 yr). All individuals involved were checked and approved by the rheumatologists in IUMS clinic. According to the patients' records erythrocyte sedimentation rate (ESR) and rheumatoid factor (RF) of each patient were determined.

Blood was collected in glass tubes containing EDTA (10mM, pH = 7.5) in order to prepare erythrocytes for flowcytometry running. Non-EDTA blood was taken and the sera were separated and used for CH50 assay. Plasma and buffy coat were separated by centrifugation at 600 *xg* for 10 min at 4°C. Erythrocytes were washed three times with PBS (0.2 M, pH = 7.4) at 1000 *g* for 2 min and the cells were suspended in PBS. Mouse Anti-Human CD59 (Serotec, CA1054) and Mouse anti-Human CD55 (Serotec, MCA1614) were used as primary antibody to stain the molecules on erythrocytes. Then F(ab')2 Rabbit Anti-Mouse IgG:FITC (Serotec,

star9B) was utilized as a secondary antibody to bind with the primary antibody and was detected with flowcytometer optic and electronic detector system. Erythrocytes were resuspended in PBS and 300UL of cells in PBS with the concentration of 1 $x10^6$ cells/ml were used in each tube, then anti-CD55 or anti-CD59 antibody was added and incubated in dark for 1 hour at room temperature. After washing the cells with PBS, FITC-labeled anti-mouse antibody was added for 1 hour at 4°C with occasional shaking. The cells were further analyzed by a flowcytometer (FACSCalibur-Becton-Dickinson). The means of fluorescence intensity of each sample indicated the amount of FITC-MAb bound to CD59 or CD55 expressed on erythrocytes. All data and histograms were recorded for final analysis. The obtained data were analyzed using a Cell Quest software package installed on the FACS system (7,15).

CH50 assay: By using a complement hemolytic fifty percent assay (CH50), the total activity of complement components C1-C9 was measured in order to measure complement activity in RA patients. The patients' sera were prepared in various dilutions and were mixed with antibody-sensitized sheep erythrocytes. Subsequently, the samples were incubated at 37°C for 1 hour and centrifuged. The quantity of erythrocyte lysis was determined by measuring the absorption of the supernatant at 540nm and the calculation was performed to assess CH50 values according to reference recommendation (16).

Calculating the means for each parameter assayed allowed the comparisons between the groups. The correlations between the biological parameters (CD59 and CD55 expression, and CH50) were evaluated by linear regression analysis using SPSS software.

RESULTS

To study the status of CD59 and CD55 expression on the erythrocytes in RA patients and normal healthy people, the specific FITC-MAbs and flowcytometry technology were applied. Cell Quest software was used and the histogram resultant analysis of $1x10^4$ cells were obtained and the mean fluorescence intensity for each independent analysis is shown in the representative graph (Fig. 1).

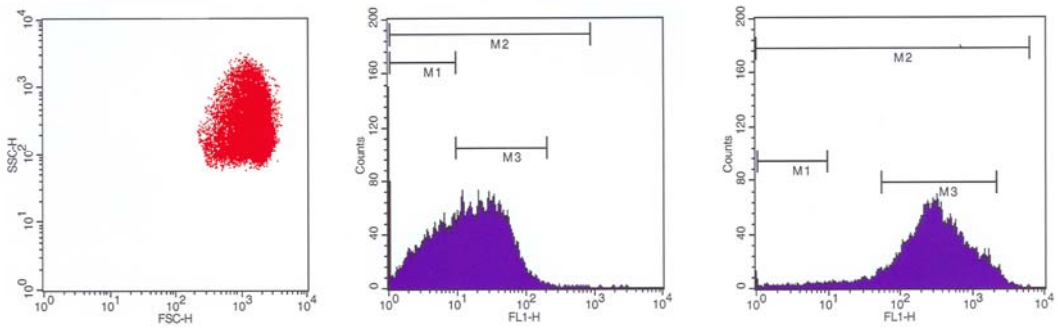

Figure 1. The representative dot plot pattern results of erythrocytes running by flowcytometry analysis (A) FSC pointed for the erythrocyte population sizes and SSC indicates the granularity of cell population. The histograms show the flourcence intensity resulting from FITC-antibody bind to primary antibody for CD55 expression (B) and CD59 expression (C) Each experiment has been carried out 2-4 times and $1x10^4$ cells were analysed in each event

The mean fluorescence intensity for CD55 was calculated to be as 27.6 ± 13.4 arbitrary unit in the patient samples and 68.5 ± 10.5 for the healthy group. CD59 mean fluorescence intensity

was 314 ± 83 arbitrary unit in patient group and 508 ± 56 in healthy volunteers. Statistical analysis showed that the difference between the means obtained from patients and healthy people was significant (P= 0.000). Regression analysis showed a significant correlation between CD55 and CD59 expression on erythrocytes in RA patients (P= 0.000, r = 0.576) (Fig. 2).

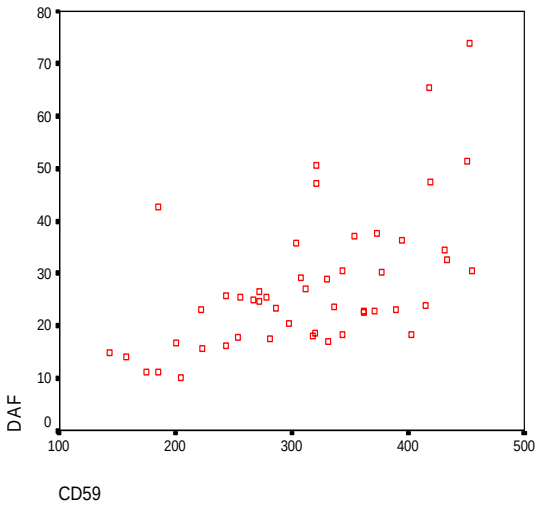

Figure 2. The association between CD55 and CD59 expression on erythrocytes taken from RA patients (r = 0.576, P = 0.000)

In addition, there was a significant difference (P = 0.000) between CH50 in patients (54.5 ± 15.5) and in healthy group (110 ± 20). The relationship between the expression of complement-regulatory proteins on erythrocytes (DAF and CD59) and CH50 activity in patients' sera was considered. However, no significant correlation was found between CH50 and either the molecule expression on erythrocytes (P = 0.143, R= -0.210 for DAF and P = 0.282, R= -0.157 for CD59). The mean value noted for ESR was 17.16 ± 11.8 mm/hours and for hemoglobin level it was 10.34 ± 4.4 gr/dl in patient group. The regression analysis was performed between both CD55 and CD59 and either the hematologic parameters (ESR and RA); however, no significant association was obtained.

Table 1. The comparative relative frequency of the parameters assayed in RA patient group and the healthy volunteer group

	RA Patient	Normal Healthy
CD 55 Expression	40	100
CD 59 Expression	60	100
CH 50 Expression	50	100

DISCUSSION

The cells of normal individuals are protected from destruction by activated complement components through the physiological protection mechanisms. These mechanisms include regulatory membrane proteins such as complement receptors (CR, MCP, decay acceleratory factor, DAF = CD55, CD59 or membrane inhibitor of reactive lysis) (2). It has been reported that the CR1 expression is down-regulated on erythrocytes in the patients with RA (17). Jones et al. considered several CD markers on neutrophils derived from synovial fluids of RA patients and the alteration in a few markers were concluded (4). This research and other similar pro-

jects led some investigators to consider marker expression in different sort of cells involved in pathogenesis of RA. Our project was defined to evaluate the regulatory protein expression in the pathogenesis of RA; therefore, the two molecules CD55 and CD59 on the erythrocytes of RA patients were considered. In addition, the complement components function was determined by CH50 assay in RA patients.

The assay showed that CD55 and CD59 receptors expression on erythrocytes from RA patients were significantly down regulated by 40% for CD55 and by 61% for CD59 compared with normal individuals. This indicates an abnormality as well as a decrease in DAF and CD55 expression in RA patients. In addition, these decreases could be the consequences of the disease progression. Regression analysis showed a significant correlation between the expression of CD59 and CD55 in RA patients (Fig. 2). This observation could lead us to conclude that deficiency of CD55 on the erythrocyte membrane may cause unrestricted complement activation and haemolysis similar to paroxysmal nocturnal hemoglobinuria (11). However, Jones et al. stated that there is no known clinical example for altered CD55 expression on erythrocytes in RA patients (4). But, similar investigation by others in other autoimmune diseases showed an association between a decreased protection of synovial cells and activation of the complement in acute and chronic arthritis (18). The present study may only show the alteration in the molecule expression, but concluding clinical symptom needs further investigation.

Arora et al. observed an inverse relationship between the relatively deficient CD55 expression on erythrocytes and in vitro complement activation (7). However, the correlation analysis between CD59 and CH50 did not reach any significant level in their report (7). Our data did not show any association between the two molecules expression on erythrocytes and in vitro complement activation assay (CH50). Regarding other autoimmune diseases, it is difficult to make similar conclusion for the pathogenesis of each autoimmune disease, for instance, according to Richaud-Patin report, while CD55 and CD59 deficiencies in primary or secondary autoimmune hemolytic anemia were determined, SLE patients exhibited a normal expression of these molecules (15). These controversial findings in known autoimmune diseases including RA need more data to be clarified. However, reduced expressions of markers on erythrocytes appear to be an acquired phenomenon in the later phases of RA process. Several explanations can be speculated to justify this phenomenon; such as a reduction in protein synthesis in original cells, blockage or masking the receptors by auto-antibodies, and proteolytic cleavage in relation to activation of complement on erythrocyte surfaces (19,20). Also to explain the reduction of expression of membrane molecules, spontaneous vesiculation which contains the membrane markers, has been suggested for acquired loss of the molecules on erythrocytes in RA patients (21). The results of the present investigation suggest that erythrocyte CD55 as well as CD59 levels may be used as a diagnostic tool in the hematologic pan tests for RA or in follow-up of the disease progression.

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