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Expression of CD55, CD59, CD46 and CD35 in Peripheral Blood Cells from Rheumatoid Arthritis Patients

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ABSTRACT

Evidences point that complement activation plays a role in rheumatoid arthritis (RA). In this context, expression of the CReg was investigated on white cells from peripheral blood of 30 RA patients and 30 healthy controls. Using flow cytometric analyses the relative fluorescence intensities (MFI) of Cregs were determined. CD59 MFI was significantly increased in RA cells comparing to controls, respectively: lymphocytes 36.8 versus 27.07; monocytes 32.0 versus 21.37; and granulocytes: 84.6 versus 66.1 ($p < 0.05$). Interestingly, no difference was observed on the MFI to CD55, CD46 and CD35 in these cells. These data indicate an overexpression of CD59 in all the peripheral blood cells of RA patients, perhaps due to an increased synthesis for compensatory mechanisms because of complement activation, inflammatory status or other factors associated with the disease.

KEYWORDS

Rheumatoid Arthritis; Complement System; Complement Regulatory Protein; Flow Cytometry

1. Introduction

Rheumatoid Arthritis (RA) is an autoimmune disease that affects 1% of the adult human population with females affected three times more than males [1]. RA is associated with persistent inflammatory polyarticular synovitis affecting mainly peripheral joints [2]. The thin synovium is normally a delicate intimal lining. In RA, it becomes thicker, resulting in swollen and tender joints because of the inflammatory infiltration that consists of macrophages, CD4+ T cells, B cells, dendritic cells, plasma cells, mast cells and granulocytes. As a result of these processes, the joint becomes deformed, unstable, inflamed, and painful, leading to great disability [3].

The complement system, when not regulated, can cause tissue damage. This condition can be induced by pro-inflammatory mechanisms, such as cytokines and chemokines. These are usually up-regulated in RA, indicating that these patients are at increased risk to damage mediated by the complement system. To counteract or contain self-damage, the complement system has a variety of regulators which can be membrane-bound or secretory proteins, which appear to be more or less efficient in distinct conditions. Normal cells resist complement-mediated lysis by several mechanisms such as specific membrane-bound proteins. Examples of these are the decay accelerating factor (CD55), the membrane inhibitor of reactive lysis or protectin (CD59), the membrane cofactor protein (CD46) and the complement receptor type I (CD35) [4,5].

Previous studies have analyzed only the expression of CD55, CD59, CD46 and CD35 in peripheral blood granulocytes and synovial fluid granulocytes [6-8], as well as CD35 expression in monocytes and lymphocytes from the peripheral blood and synovial fluid of patients with RA [8-10]. The aim of this study is to analyze the expression profile of CD55, CD59, CD46, and CD35 complement regulatory (CReg) proteins on the surface of leukocytes by flow cytometry in blood donors and pa-

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tients with RA.

2. Materials and Methods

2.1. Subjects

Thirty consecutive patients that fulfilled the American College of Rheumatology classification criteria [11] for RA were included. The controls were blood donors without history of disease and were matched by age and sex. The RA patients were seen during their routine follow-up visits in the RA clinics of the Hospital de Clínicas de Porto Alegre. The exclusion criteria were concomitant presence of leukaemia, primary lymphoproliferative diseases, or overlap with an autoimmune disease. This study was performed with approval of the ethics committee of the Hospital de Clínicas de Porto Alegre/ Brazil, and all subjects gave their written informed consent.

2.2. Flow Cytometric Analysis of CD55, CD59, CD46 and CD35 Membrane in Leukocytes

The flow cytometric analysis was processed as reported elsewhere [12]. Briefly, 100 μL of whole blood were stained with Fluorescein isothiocyanate (FITC) or Phycoerythrin (PE) conjugated monoclonal antibody (CD55 PE, CD59FITC, CD35PE and CD46FITC (BD Biosciences, San Diego, CA, USA)). After incubation, 1.0 mL of FACSlyse (BD Biosciences, San Diego, CA, USA) was added and lysis was allowed for 10 min at room temperature. Samples were washed once and re-suspended in PBS.

Cells were analyzed on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences, San Diego, CA, USA). Membrane fluorescence intensity was estimated by the relative mean fluorescence intensity (MFI). Cells labeled with isotype-controls were used to set the fluorescence thresholds for positivity fluorescence.

2.3. Statistics

Varibles with normal distribution were presented with mean and standard deviation. The statistical significance of the differences observed between the distinct subsets of leukocytes (means) were calculated using Student's t-test. Correlation of disease activity (DAS28) and CReg expression was evaluated using Spearman correlation coefficient. p values < 0.05 were considered to be associated with statistical significance.

3. Results

The description of the 30 patients and 30 healthy controls and the treatments are summarized on **Table 1**. Twelve of the 30 RA patients (40%) had leukocytosis whereas seven of the 30 RA patients (23.3%) had monocytosis, four (13.3%) had granulocytosis and two (6.7%) had lymphocytosis. Seven of the 30 RA patients (23.3%) showed lymphopenia.

To evaluate the proportion and MFI of CD55, CD59, CD46 and CD35 cells in RA patients and healthy controls, CellQuest software was used and the cells were performed as previous work [12].

Flow Cytometry Analyses from the Blood Cells

The CD59 MFI in RA cells was significantly increased compared to controls cells: granulocytes (84.60 vs. 66.10), monocytes (32.00 vs. 21.37) and lymphocytes (36.8 vs. 27.07) (**Figure 1**).

The proportions of CD59+ (91.84%), CD46+ (99.78%), CD35+ (98.50%) in RA monocytes were also significantly increased. Together with the proportions of CD59+ (91.84%) in RA lymphocyte. Meanwhile, CD55, CD46 and CD35 MFI did not show significant differences between RA and controls, as summarized on **Table 2**.

Regarding association with disease activity (DAS28), we observed increased CD59 expression on lymphocytes, monocytes and granulocytes from patients with higher activity (data not shown), however there was no significance correlation (CD59 MFI Lymphocytes, $p = 0.288$; CD59 MFI Monocytes, $p = 0.757$ and CD59 MFI Granulocytes, $p = 0.208$).

4. Discussion

In this study we found a CD59 increased expression in all cells in the peripheral blood of patients with rheumatoid arthritis: on lymphocytes, monocytes and granulocytes. In contrast with our findings, Jones *et al.* [7] observed no difference between MFI of CD59 on granulocytes from peripheral blood of RA patients compared with healthy controls. However they evaluated a rather small number of patients (n = 18), and the study might not have had enough power to detect a difference. These authors did not evaluate monocytes and lymphocytes.

Furthermore, Konttinen *et al.* [13] have described that RA synovial cells do not express CD59, and that CD59 expression is weak in many synovial stromal and endothelial cells. In addition, CD59 expression seems to be partially lost in many acute arthritis in these cells. The finding of a lack of CD59 expression in synovial cells in RA and acute arthritis might imply in an inefficient control of MAC activation in synovial tissue. This could predispose to synovial cell damage or to various stimulatory effects by MAC. Factors inducing this low expression are not known.

In another study, Crockard *et al.* [6] have noted that CD35 expression was upregulated on synovial fluid gra-

Table 1. Description of RA patients and healthy controls.

	RA patients	Healty controls	p value
	(n = 30)	(n = 30)	
Sex			
Man % (n/total)	16.7 (5/30)	16.7% (5/30)	
Woman % (n/total)	83.3 (25/30)	83.3% (25/30)	
Age (year)	53 ± 12	53 ± 12	0.960
DAS-28[a]			
Remission % (n/total)	30 (9/30)	-	
Low activity % (n/total)	20 (6/30)	-	
Moderate activity % (n/total)	16.6 (5/30)	-	
High activity % (n/total)	16.6 (5/30)	-	
Missed % (n/total)	16.6 (5/30)	-	
Treatments			
BT[b] + DMARDs[c] % (n/total)	13.3 (4/30)	-	
BT + DMARDs + Corticosteroids % (n/total)	3.3 (1/30)	-	
BT + corticosteroids % (n/total)	3.3 (1/30)	-	
DMARDs + corticosteroids + AINEs[d] % (n/total)	16.6 (5/30)	-	
DMARDs + corticosteroids % (n/total)	26.6 (8/30)	-	
DMARDs % (n/total)	30 (9/30)	-	
AINEs % (n/total)	3.3 (1/30)	-	
Leukocytes ($\times 10^3/\mu L$) (mean ± SD)	8.102 ± 2.836	7.300 ± 1.858	0.201
Monocytes ($\times 10^3/\mu L$)	0.769 ± 0.339	0.599 ± 0.180	0.020*
Granucytes ($\times 10^3/\mu L$)	4.843 ± 2.119	4.185 ± 1.362	0.159
Lymphocytes ($\times 10^3/\mu L$)	2.262 ± 1.009	2.266 ± 0.756	0.989

*Statistically significance, $p < 0.05$, [a]DAS28: Disease Activity Score 28, [b]BT: Biologic Therapy, [c]DMARDs: Disease-Modifying Antirheumatic Drugs, [d]AINEs: non-steroidal anti-inflammatory drugs.

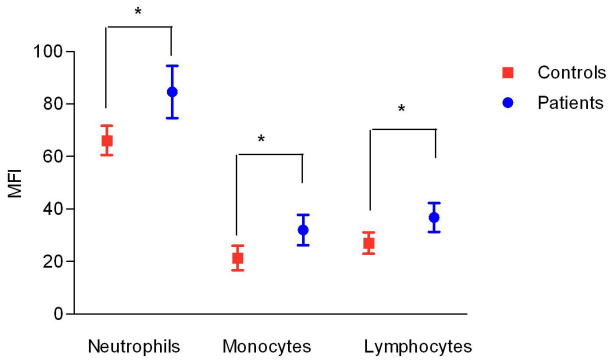

Figure 1. Expression of CD59 in peripheral blood cells in RA patients (patients) and healthy control subjects (controls). Data shown as mean and stard deviation. (*) = p < 0.05 t test.

nulocytes compared with peripheral blood granulocytes. Indeed, McCarthy *et al.* showed that when granulocytes are activated *in vitro* CD35 expression is increased. Concerning monocytes, Gadd *et al.* [10] found CD35 to be upmodulated in cells from synovium and blood. Also, Torsteinsdóttir *et al.* [9] reported that the expression of CD35 in RA monocytes was elevated, in accordance to our data, although we did not found a significant difference. On the other hand, Jones *et al.* [7], observed a significant reduction in the expression of CD35, CD55 and CD46 on peripheral blood granulocytes in RA patients. It has been discussed that the difference in the expression of these molecules leads to increased adhesiveness, resistance to complement and an increased capacity of neutrophils to remove immune complexes. We did not observe any difference in the proportion and MFI for CD55, CD46 and CD35 in peripheral blood granulocytes.

Moreover, we found that the absolute value of monocytes with positive CD59 was significantly higher than controls (data not shown). This increase of expression of

Table 2. Expression of the CReg in the patients and controls.

		Patients (n = 30) MFI (Mean ± SD)	Controls (n = 30) MFI (Mean ± SD)	t-test ($p < 0.05$)
CD55	*Lymphocytes*	353.43 ± 116.42	294.67 ± 132.26	0.0730
	Monocytes	1058.00 ± 321.80	1096.70 ± 273.48	0.6177
	Granulocytes	605.33 ± 166.83	605.33 ± 184.71	1.000
CD59	*Lymphocytes*	36.80 ± 14.78	27.07 ± 10.90	0.0054*
	Monocytes	32.00 ± 15.51	21.37 ± 12.42	0.0049*
	Granulocytes	84.60 ± 26.65	66.10 ± 14.93	0.0018*
CD46	*Lymphocytes*	69.03 ± 13.23	69.87 ± 16.38	0.8292
	Monocytes	67.07 ± 10.99	66.07 ± 10.69	0.7221
	Granulocytes	97.97 ± 25.29	101.87 ± 16.75	0.4846
CD35	*Lymphocytes*	104.13 ± 115.22	96.43 ± 93.28	0.7771
	Monocytes	149.97 ± 98.72	127.37 ± 80.48	0.3353
	Granulocytes	128.23 ± 72.23	110.40 ± 58.82	0.2989

Subtitle: [a]MFI: mean fluorescence intensity. [b]Percentage: percentage of positive cells for the studied marker.

this regulatory protein could be due to the monocytosis present in RA patients ($p = 0.02$, data not shown) and we do not find any other report in the literature. The reason by which these CReg are higher expressed in monocytes from RA patients, and the possible correlation with monocytosis need to be further explored.

About CD46 we did not observe any statistically significant difference in CD46 expression in granulocytes, monocytes and lymphocytes from peripheral blood of patients with RA compared with healthy controls. By contrast, Jones *et al.* [7] found a statistically significant decrease in the expression of CD46 on granulocytes from peripheral blood. We did not find another study for this protein in peripheral blood leukocytes in RA to elucidate these opposite findings. Recent work has suggested that CD46 can play a role in modifying adaptative immunity, suppressing T-regulatory cells and as a scavenge of damaged cells [14].

The mechanisms involved in the upregulation of CD59 expression are still unclear. CD59, a 18 kDa glycoprotein anchored to the cell membrane by glycosylphosphatidylinositol (GPI) linkage, is constitutively expressed on haematopoietic and resident tissue cells throughout the human body. The main function of CD59 derives from its capacity to interact with the C8 and C9 components of the cytolytic MAC to block the conversion of C9 from a hydrophilic to an amphipathic molecule, thus preventing insertion of C9 into the membrane [15]. It is suggested that CD59 may be involved in several immunological phenomena, such as: interaction with proteins of lymphocytes, monocytes and granulocytes, necessary for activation of these cells [16,17]; exert synergic function in T cell adhesive interactions [18]; play a role in cell interaction between monocytes and T cells [19]; and may suppress the production of pathogenic antibodies [20].

In summary, we believe that our findings may help explain the mechanisms of CRegs expression modified in RA patient. And also we did not found any other report at the literature showing CD59 increase in all peripheral blood cells in RA. Several explanations can be proposed to explain to the CD59 increased expression in leukocytes in RA: 1) increased synthesis for compensatory mechanisms because of complement activation; 2) leukocytes activation and/or 3) disease activity. Whether these changes are contributory to the disease or merely a consequence of the chronic inflammatory state remains unclear. However, the data suggest that CReg might be working systemically to suppress the disease activity associated with uncontrolled complement activation in RA.

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