Phosphatidylserine induced up-regulation of Cluster Differentiation 36 and 47 on red cell membrane

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Abstract: **Aim:** The purpose of this research was to study the expression of CD36 and CD47 on red cell membrane following perturbation of red cell membrane with phosphtidylserine (PS**). Materials and Methods:** Blood was drawn from 100 healthy adults; Incubation of RBCs with different serial concentrations of PS was carried out at 37°C for 72 hrs. All tubes were washed followed by addition of CD36-FITC and CD47-FITC antibodies, Cells were analyzed for the expression of CD markers using the FACS analyzer. Data were analyzed by using Statistical Package for Social Science (SPSS) version 21 and Microsoft Excel 2013**. Results**: were obtained by using student T .test. Result: CD36 expression is directly proportional to PS serial dilutions; however the expression of CD47 is raised in the last three higher concentrations. **Conclusion:** We conclude that CD47 has been shown to inhibit phagocytosis of erythrocytes by macrophages of the reticuloendothelial system, whilst CD36 responsible of recognition of red cell by macrophage and accelerating engulfing process PS induced up regulation of CD36 and CD47 which may encourage their conversed action and participate in keeping red cell as much as normal in diseases associated with high concentration of PS.
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1. Introduction

The human RBC (RBC) membrane consists of lipids (41%), proteins (52%), and carbohydrates (7%). [1, 2] In average, there are about 5.2 mg membrane lipids per ml of packed RBCs or approximately 5.2 × 10-13 g/cell. Membrane lipids can be classified into three classes: neutral lipids (25.2%), phospholipids (62.7%) and glycosphingolipids (about 12%). Neutral lipids of human RBCs represent cholesterol almost exclusively [3, 4]. The ratio of cholesterol to phospholipid is about 0.8. Phospholipids consist of sphingomyelin (SM, 26%), and glycerophospholipids. [5]

The lipid composition of RBC membrane is rather stable and only alters with diet to a limited extent [6, 7]. This is due to the lack of de novo synthesis of phospholipids in the mature RBC. Limited alterations of the fatty acid composition by diet result from the exchange of phospholipids, primarily PC, between plasma lipoproteins and the cell membrane, as well as the exchange of fatty acids [8, 9]

CD36 is a multi-functional molecule. It has independent binding sites for different classes of ligands Such as modified phospholipids, thrombospondins, and free fatty acids. This enables CD36 responsible for several different cellular processes depending on the nature of the ligand and the type and location of the cell on which it is expressed. [10] CD36 also functions as an adhesion molecule. [11]

CD47 is a 47–52 kDa transmembrane glycoprotein with a ubiquitous expression profile in human tissues that includes erythrocytes [12]. On mature erythrocytes, which lack integrins, CD47 appears to mediate cell-cell interactions with SIRP- of splenic macrophages. This association is thought to inhibit a phosphorylation cascade that blocks phagocytosis and prevents erythrocyte clearance from the circulation. [13] The purpose of this research was to study the expression of CD 36 andCD47 on red cell membrane following perturbation of red cell membrane with phosphtidylserine.

2. Material and Methods

Blood was drawn by venipuncture into evacuated tubes containing EDTA anticoagulant. Nor mal samples were drawn from 100 healthy apparently adult male volunteers, his lipid profile within normal range. Samples were classified into three categories:

Ten participants as control group without adding any reagent and other two groups 45 participants for each were tested for CD36 and CD47.

Stock concentration (1000 mg/ml) of L-a-lysophosphatidylserine (commercial name of phosphtidylserine PS) was dissolved in 4 ml RPMI medium under sterile conditions followed by

vortexing for 30 seconds. In order to prepare serial dilution of PS from 100 mg/dl up to 1000 mg/dl. 10µl of each dilution was added to 1000µl of washed red cells.

Incubation of RBCs with different concentrations of phophotidylserine was carried out at 37°C in siliconized or polyethylene flasks, using a metabolic shaker, for periods of up to 72 hr. sterile conditions were maintained and were confirmed by culture. Penicillin (100 units/ml) and streptomycin (0.1 mg/ml) were used in some of the incubation and had no effect on the results. Sufficient glucose was added to provide a total concentration of 12 umoles/ml of blood. Then 45 tubes were labeled with CD36 500µl washed red cells were added. Same procedure was done to CD47.

All tubes were washed again for three times in phosphate buffer saline (PBS) and resuspended in 4500µl PBS, followed by addition of 20µl of CD36-FITC antibodies to second group and CD47-FITC antibodies for third group and mixed gently (except the control tube). All tubes were incubated at 4ºC for 30 minutes and washed 3x times with PBS, and all red cells were suspended in 1ml of PBS. Cells were analyzed for the expression of CD markers using the FACS analyzer.

Data analysis:

Data were analyzed by using Statistical Package for Social Science (SPSS) version 21 and Microsoft Excel 2013. Results were obtained by using student T test.

Ethical approval

Ethical clearance was obtained from the Ethical Committee Board of Tropical Medicine Institute. The verbal of the consent was taken from selected individual after being informed with all objectives of the study and its health impact in the future.

3. Results

A total of 100 individuals participated in the present study. Red cell tubes were treated with serial concentration of PF from 100mg/dl-1000mg/dl except the control sample. In figure 1 as control (without PS) and figure 2 (treated with PS) the flow cytometer histograms show distribution of red cell label with FITC-CD36.

In figure 3 as control (without PS) and figure 4 (treated with PS) the flow cytometer histograms show distribution of red cell label with FITC-CD47. Table 1 shows CD36 and CD47 expression means in serial concentrations of PS, CD 36 has high significant expression with the increasing the concentrations of PS while the CD47 expression is significant in last 3 concentrations of PS and CD expression doesn't affect with low concentration of PS data were analyzed by using student t test.

In figure 5 the CD36 expression is directly proportional with PS serial dilutions, but in figure 6 the expression of CD47 increased proportionally to last three concentrations.

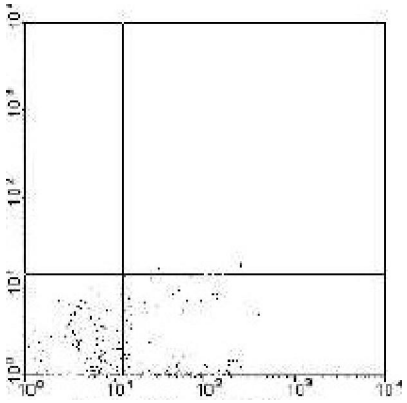

Figure 1: Flow cytometer histogram shows distribution red cell label with FITC-CD36 for control sample (without PS).

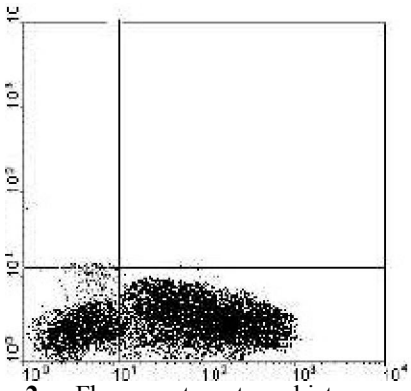

Figure 2: Flow cytometer histogram shows distribution red cell label with FITC-CD36 and treated with PS.

Table 1: CD36 expression means in serial concentrations of PS were added to washed red cells and treated with FITC-CD36 (no= 45) and FITC; CD47 (n=45) and statistically analyzed by using Student T test.

Serial concentrations of PS	CD36 Mean ± STD	CD47 Mean ± STD
100 mg/dl	1.20 ± 0.88	0.17 ± 6.61
200 mg/dl	2.58 ± 1.81	0.44 ± 5.91
300 mg/dl	8.30 ± 1.28*	0.12 ± 3.90
400 mg/dl	9.97 ± 3.77**	4.07 ± 5.82
500 mg/dl	14.74±1.10 **	3.54 ± 6.80
600 mg/dl	17.29 ± 1.47**	4.47 ± 6.44
700 mg/dl	19.06 ± 1.40**	5.30 ± 8.31
800 mg/dl	22.39 ± 1.26**	18.19±6.07**
900 mg/dl	26.08 ± 1.48**	23.52 ± 6.13**
1000 mg/dl	35.03 ± 5.31**	29.69 ± 7.42**

*P≤ 0.05 **P ≤ 0.001

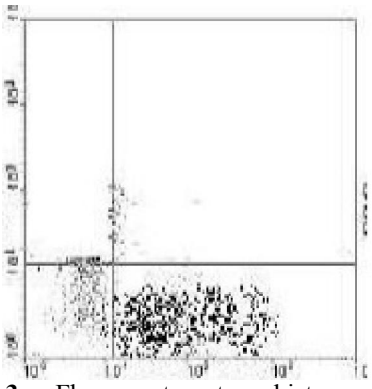

Figure 3: Flow cytometer histogram shows distribution red cell label with FITC-CD47 for control sample (without PS).

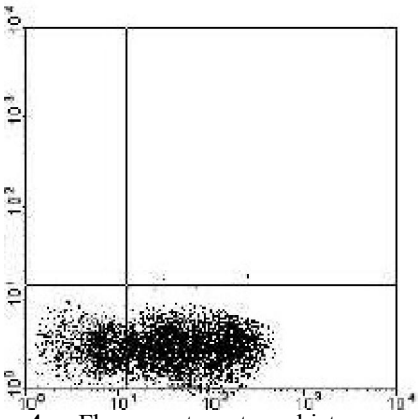

Figure 4: Flow cytometer histogram shows distribution red cell label with FITC-CD47 and treated with PS

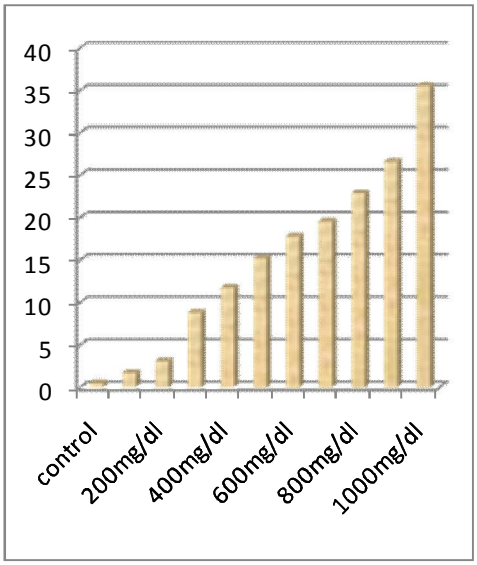

Figure 5: shows the correlation between serial concentrations of PS and CD36 expression on red cell surface.

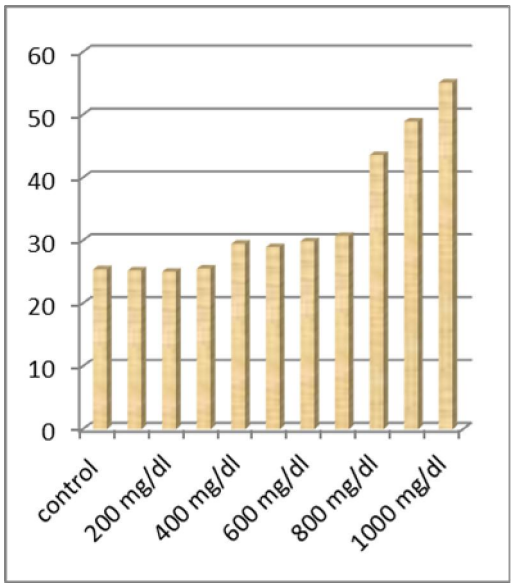

Figure 6: Shows the correlation between serial concentrations of PS with CD47 expression on red cell surface.

4. Discussions

Phospholipids are distributed asymmetrically between the two faces of the plasma membrane [14]. In particular, PS is nearly absent from the extracellular face of the plasma membrane in resting cells. Membrane phospholipid asymmetry is maintained by the aminophospholipid translocase, an ATP-dependent enzyme that transports PtdSer and phosphatidylethanolamine from the outer face to the inner face of the plasma membrane. [15] In the present study red cell membranes was perturbed by incubation of red cell in different concentrations of PS and measure the changing in expression of CD36 and CD47 on red cell membrane. A number of reports have indicated that some exchange of erythrocyte lipid with corresponding plasma compounds occurs [16]. CD 36 expression is significantly increased in numbers and directly proportional to the concentration of PS in our study as shown in table 1and figure 5. CD36 responsible for several different cellular processes depending on the nature of the ligand and the type and location of the cell on which it is expressed. On phagocytes, CD36 functions as a scavenger receptor helping in recognition and internalization of apoptotic cells. [17] Expression of CD36 in red cell CD36 is also thought to play a major role in infection with Plasmodium falciparum malaria. Most patient isolates of P falciparum have the capacity to adhere to CD36. Adherence of infected cells to CD36 on endothelial cells24 is thought to contribute to the sequestration of mature infected cells in the microvasculature, thus preventing their destruction in the spleen. Adherence

of infected cells to CD36 on monocytes activates these monocytes15 and thus may contribute to the immunopathology of 1nalaria. [18]

In our study the expression of CD47 wasn't affected with low concentrations of PS while in high concentrations CD47 was increased significantly as shown table 1 and figure 2. CD47 has been shown to inhibit phagocytosis of erythrocytes by macrophages of the reticuloendothelial system. CD47 exerts its inhibitory effect through binding to SIRP on the macrophage, which induces inhibitory signaling by the immunoreceptor tyrosine based inhibition motifs (ITIMs) residing in the cytoplasmic tail of SIRP. On ligation of SIRP by CD47, the SIRP ITIMs recruit and activate tyrosine phosphatases SHP-1 and SHP-2, and this regulates, generally in a negative fashion, downstream signaling pathways and effector functions. The inhibitory effect of CD47 on erythrocyte clearance can be illustrated by transfusion of CD47-deficient erythrocytes into a wild-type recipient, which leads to rapid phagocytosis of the CD47-deficient erythrocytes by red pulp macrophages in the spleen. Thus, phagocytosis of erythrocytes is supposed to be the result of "eat me" signals already present that override the inhibitory signal of CD47 [17]. CD47 interaction is well known for its ability to inhibit phagocytosis of CD47-expressing red cell and this process is promoted by increasing of PS level, in addition to that red cell CD36 receptor is recognized by macrophage and engulfing process takes place through, so that CD36 and CD47 induce different mechanisms on red cell behavior, obviously observed in high concentrations of PS which is associated with increasing in expression of two receptors and they have reverse action on red cell which may lead red cell to survive nearly to normal life span even in disease associated with high concentration of PS.

Conclusion

We conclude that CD47 has been shown to inhibit phagocytosis of erythrocytes by macrophages of the reticuloendothelial system, However CD36 responsible of recognition of red cell by macrophage and accelerating engulfing process. PS induced up regulation of CD36 and CD47 which may encourage their conversed action and participate in keeping red cell as much as normal in diseases associated with high concentration of PS.

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Conflict of Interests

The authors have declared that no conflict of interest exists.

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