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AN INVESTIGATION INTO THE PRESENCE OF COMPLEMENT PROTEINS ON ERYTHROCYTE VESICLES IN SICKLE CELL DISEASE

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INTRODUCTION

Latest insights into the pathophysiology of sickle cell disease (SCD) indicate that inflammation and hypercoagulability caused in part by increased phosphatidylserine exposure and extracellular vesiculation by sickle erythrocytes[(1,2)].

Studies have identified some of these vesicles as those released from reticulocytes in the process of maturation to erythrocytes and are termed 'inside-out' autophagic vesicles as they are the result of fusion of endocytosed plasma membrane with autophagic endosomes and stain positively for intracellular glycophorin A (GPA) epitope BRIC163 [(3)]. Further work has shown that some of these vesicles are also positive for an extracellular GPA epitope (BRIC256) raising the possibility that the vesicle membrane may have become permeabilized.

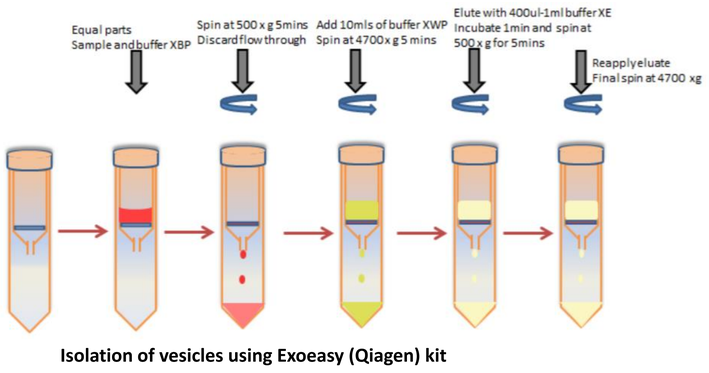
One possible mechanism to explain permeabilization is the action of the membrane attack complex (MAC) of the complement system .

AIM

The aim of the project was thus to investigate whether complement proteins are present on erythrocyte derived vesicles during extrusion from the cell and while circulating freely in plasma.

METHOD

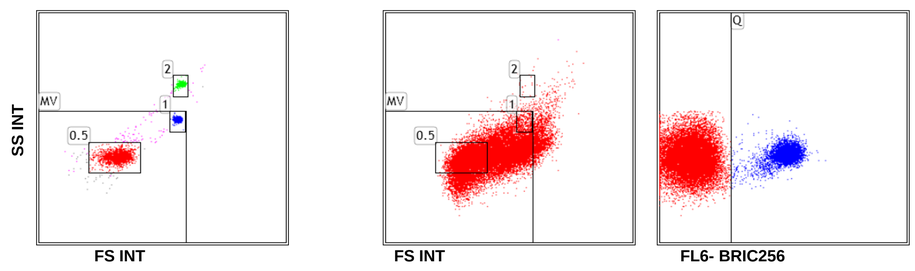
Vesicles were isolated from SCD plasma (n=2) and healthy control plasma (n=1) using membrane-based isolation technique and fluorescence-activated cell sorting (FACS) before being studied by flow cytometry using antibodies against complement proteins C3, C4, C3c and C3d and intracellular and extracellular epitopes of GPA. Cultured reticulocyte, sickle erythrocytes and healthy control erythrocytes were analyzed by confocal microscopy



Isolation of vesicles using Exoeasy (Qiagen) kit

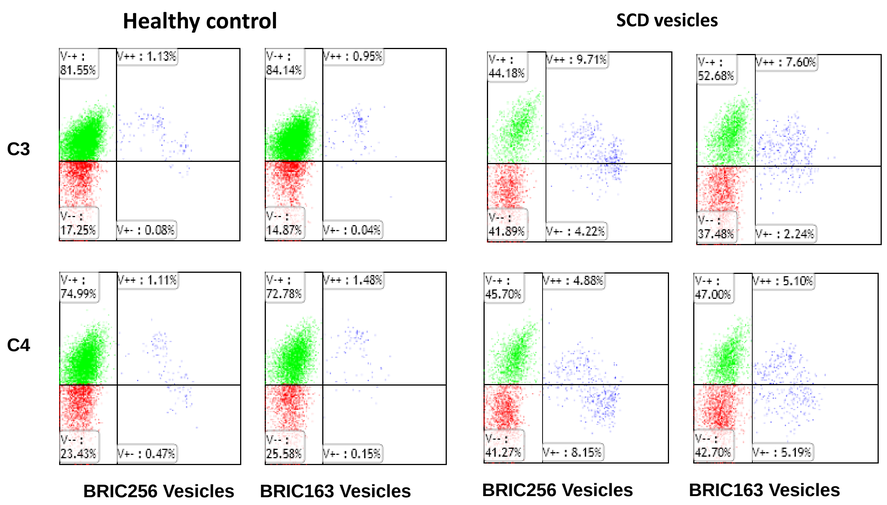
RESULTS

GATING STRATEGY

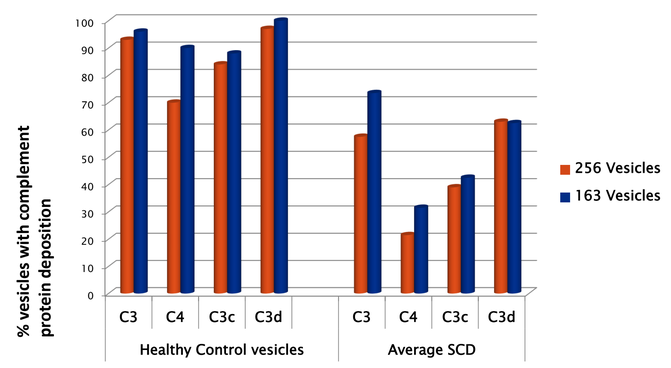


A gating strategy was established using size beads of 0.5, 1 and 2µm. A gate was created around 1µm and smaller size beads and was applied to flow cytometry analysis of isolated and sorted vesicles.

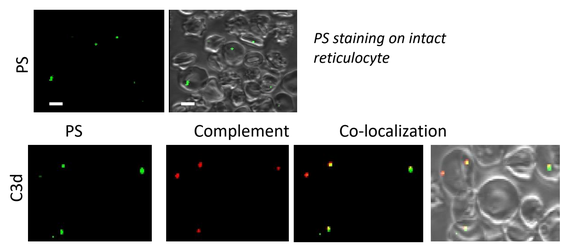
COMPLEMENT ANALYSIS OF SORTED VESICLES

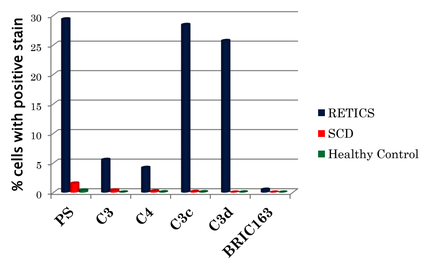


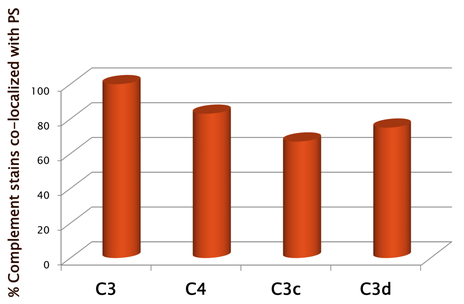
There were more vesicles extracted from the SCD plasma compared to healthy control. However, more vesicles from healthy plasma were positive for complement proteins C3 and C4



CONFOCAL MICROSCOPY OF INTACT RETICULOCYTES, SICKLE RED CELLS AND HEALTH CONTROL RED CELLS STAINED WITH PHOSPHOTIDYLSERINE (PS), COMPLEMENT PROTEINS C3, C4, C3d and C3c with Co-localization analysis







CONCLUSIONS

Results show complement protein deposition on both vesicles and intact red cells. As expected, fewer vesicles was isolated from healthy control plasma when compared with sickle plasma.

Complement proteins deposition was higher in vesicles extracted from healthy plasma compared to sickle vesicles. This might be explained by relative complement deficiency that has been demonstrated in sickle cell patients[(5)].

Confocal microscopy showed heavy PS and complement deposition on reticulocytes compared with red cells from both sickle patient and healthy control. Furthermore, there was high percentage of co-localization of phosphotidylserine with complement proteins suggesting complement proteins may be involved in vesicle extrusion through the formation of MAC complex.

Study was limited by small sample size. Due to absence of triple staining with BRIC163, BRIC256 and complement proteins, study could not determine the exact relationship between complement proteins and dual positive erythrocyte vesicles.

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