PHOSPHATIDYLSERINE EXPOSURE AND RED CELL VIABILITY IN RED CELL AGING, STORAGE, AND IN HEMOLYTIC ANEMIA

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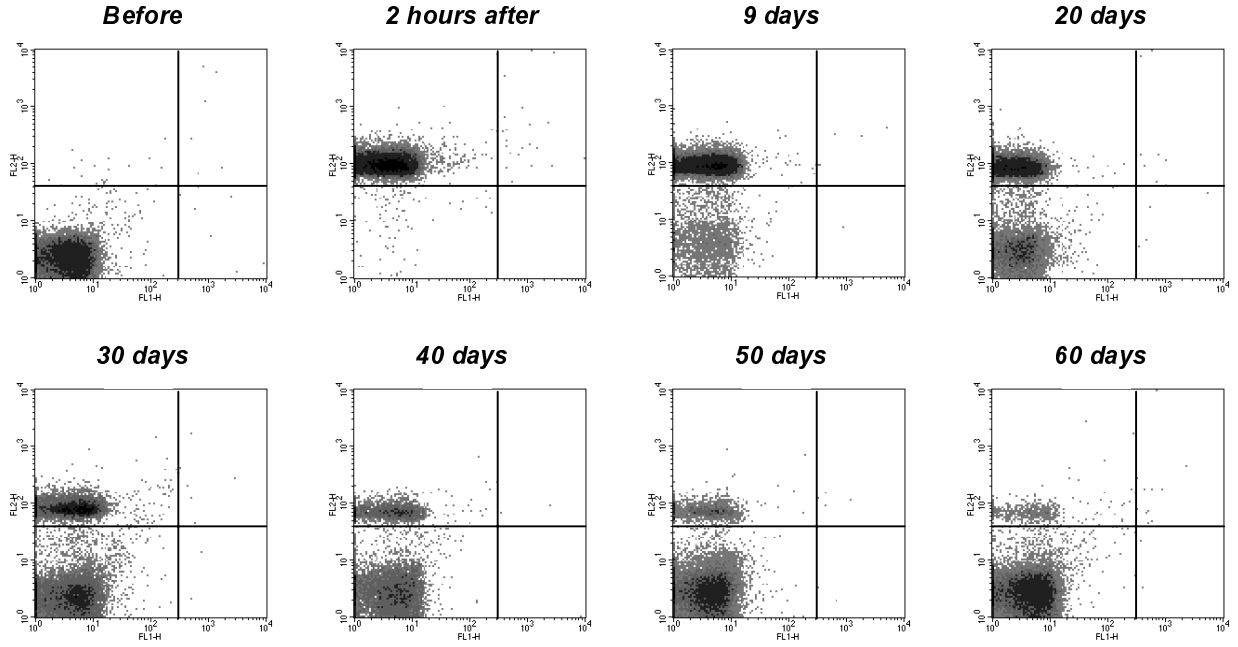
ABSTRACT

Phosphatidylserine (PS) normally localizes to the inner leaflet of cell membranes but becomes exposed in abnormal or apoptotic cells, signaling macrophages to ingest them. Along similar lines, it seemed possible that the removal of red blood cells from circulation because of aging, the storage lesion, or various hemolytic anemias might be triggered by PS exposed on the cell's outer surface.

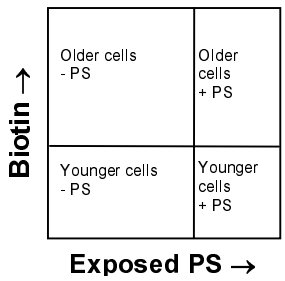
To investigate the role of PS exposure in normal red cell aging, we used N-hydroxysuccinimide-biotin to tag rabbit red cells *in vivo*, then used PE-streptavidin to label the biotinylated cells, and annexin V-FITC to detect the exposed PS. Flow cytometric analysis of these cells drawn at 10 day intervals up to 70 days after biotinylation indicated that older, biotinylated cells expose more PS. Furthermore, our data match a simple model of red cell senescence that assumes both an age-dependent destruction of senescent red cells preceded by one to six hours of PS exposure and a random destruction of red cells. Using this model, we demonstrated that the exposure of PS parallels the rate at which biotinylated red cells are removed from circulation. On the other hand, using an annexin V-FITC label and flow cytometry demonstrates that exposed PS does not cause the blood storage lesion, which refers to the removal of many stored red cells soon after transfusion. Normal human blood stored in CPDA-1 or ACD-A for up to 5 weeks has only a small proportion (under 3.5%) of red cells with exposed PS, a proportion that remains small even after six hours of metabolic incubation to simulate the return of stored cells to the circulation after transfusion. Exposed PS also does not explain the reduced red cell life span of hemolytic anemia patients, with the possible exception of those with unstable hemoglobins or sickle cell anemia. Thus, in some cases PS exposure on the cell surface may signal the removal of red blood cells from circulation, but in other cases some other signal must trigger the sequestration of cells.

NORMAL RED CELL AGING

Exposure of PS significantly increases as red cells age, and this exposure parallels the rate at which older cells are removed from the circulation. This evidence supports a model of red cell senescence in which aged red cells expose PS, signaling macrophages to remove them from the circulation.

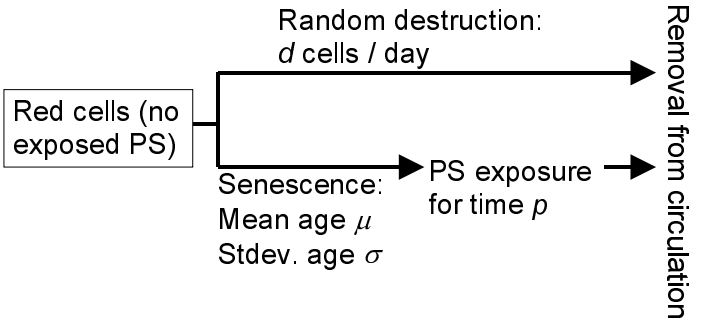


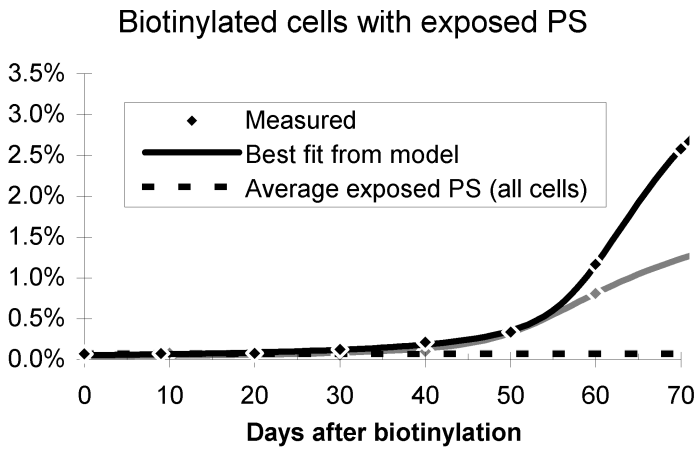
Density plots of FL1 (FITC fluorescence, exposed PS) vs. FL2 (PE fluorescence, biotin) in red cells before and after irreversible *in vivo* biotinylation (Rabbit #2). The quandrants indicate cutoffs for determining biotinylation and exposed PS. Notice that biotinylated cells steadily decrease in number after biotinylation and tend to have more exposed PS than non-biotinylated cells.

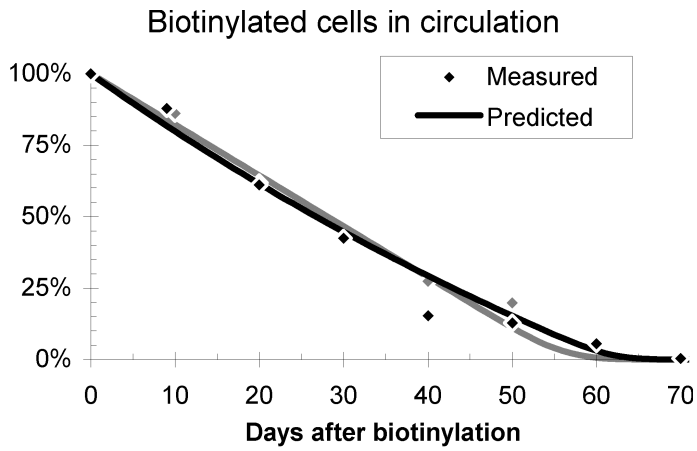


Model of red cell aging shown to the right. Fitting the parameters of the model to all of the experimental data produces the following results:

	Rabbit #1	Rabbit #2
Random death (*d*)	1.1% / day	1.4% / day
Senescence: Mean (μ)	65 days	62 days
Stdev. (σ)	4 days	3 days
PS exposure time (*p*)	1 – 6 hrs.	1 – 6 hrs.





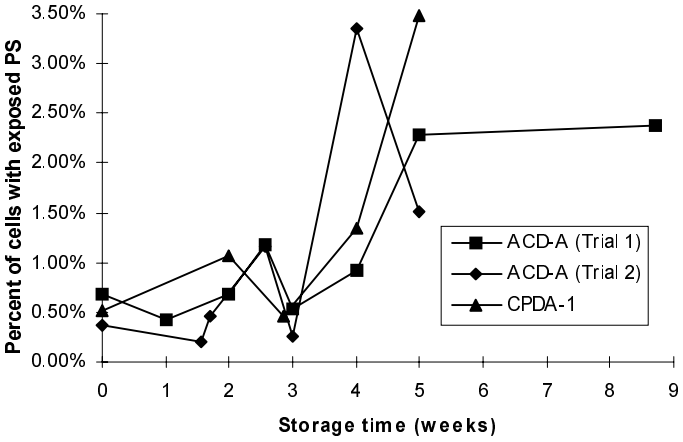


Graphs on the left show that older, biotinylated cells expose more PS in both Rabbit #1 (gray points and curves) and Rabbit #2 (black points and curves). The parameters for our model of red cell senescence were chosen to provide a least-squares fit to these data on exposed PS in biotinylated cells (r^2>0.98).

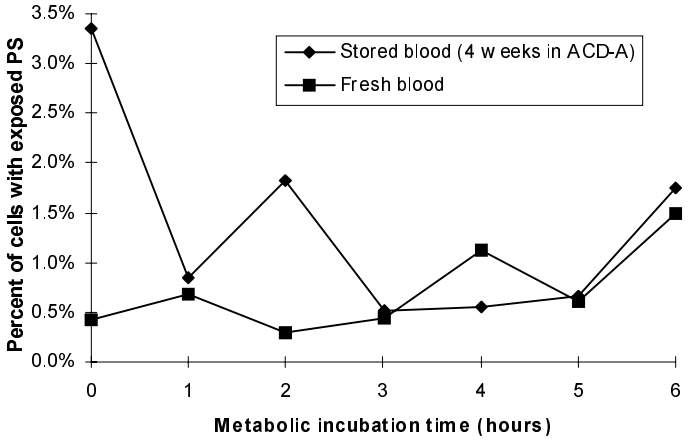
Graphs on the right show that the model correctly predicts the rate at which biotinylated cells are removed from the circulation (r^2>0.98). This supports our model, which assumes that the amount of exposed PS is proportional to the rate of senescent death.

BLOOD STORAGE

The reduced viability of red cells transfused after storage is not associated with increased PS exposure.



Exposed PS as a function of human blood storage time in ACD-A and CPD A-1. The percent of cells with exposed PS increases after 3 to 4 weeks of storage, but not enough to cause the storage lesion.



Exposed PS in human red cells as a function of hours of metabolic incubation. PS exposure remains low during incubation.

HEMOLYTIC ANEMIA

The reduced red cell life span of most hemolytic anemia patients is not associated with exposed PS.