Mechanism of Microaggregate Formation in Stored Blood

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BLOOD STORED at 4° C. in ACD solution has been shown to develop microaggregates composed primarily of platelets and fibrin.[7,10] This debris in transfused blood has been shown to produce changes in pulmonary hemodynamics in animals[3,6] and has been implicated in the pathogenesis of post-traumatic pulmonary insufficiency in man.[4,8,9]

The present studies were undertaken to define more clearly the mechanism by which these microaggregates are formed. It is directed primarily at studying evolutionary changes in platelet function and fibrinogen composition of stored human blood.

Materials and Methods

Ten units of fresh human blood were collected in the customary fashion in plastic bags containing ACD solution and stored at 4° C. Blood was studied on the day of collection, each subsequent day for 5 days and at 3-day intervals thereafter.

Studies included the following:

Hematocrit (microhematocrit method)

Platelet Count (phase contrast microscopy)

Platelet Aggregation (percentage decrease in optical density 4 minutes after addition of 0.1 uM ADP solution)[2]

Screen Filtration Pressure (both quantitative and qualitative as previously described)[5]

Serum Fibrinogen[12]

Fibrinogen content of microaggregate material. This was performed by adding 25 ugm of ^{125}I tagged 90% clottable human fibrinogen to each bag of freshly drawn blood. Blood was counted before filtration through the SFP apparatus with a Scintillation counter. The SFP and debris weight was then determined. The SFP screen was removed, weighed and counted and the filtrate volume was measured and counted. Fibrin on the serum was expressed as counts/gm of debris.

Results

Hematocrit remained quite stable throughout the 21 days of storage (Fig. 1). Platelet count rose initially, then fell progressively from 2 through 15 days, remaining constant for the remaining 6 days of study (Fig. 2). Platelet aggregation increased slightly during the first 3 days of storage, stabilized through day 10 and fell during the remainder of the storage period (Fig. 3). Debris accumulation began at day 2 and increased to day 5, remained constant through day 10, then increased rapidly during the next 11 days of storage (Fig. 4). Scintillation counts remained constant during the first 10 days of storage, then increased rapidly, paralleling the increase in debris weight for the remainder of the storage period (Fig. 5).

Discussion

The small but significant initial rise in platelet count may be explained by de-aggregation of small platelet aggregates formed during the process of blood drawing. Increase in platelet propensity to aggregate parallels the early formation of microaggregates detected by SFP from storage days 2 through 5. Platelet count and aggregation remained stable from days 4 through 10. At the same time, no further microaggregate accumulation was noted suggesting that the initial microaggregate formation was due primarily to platelets. This is further sup-

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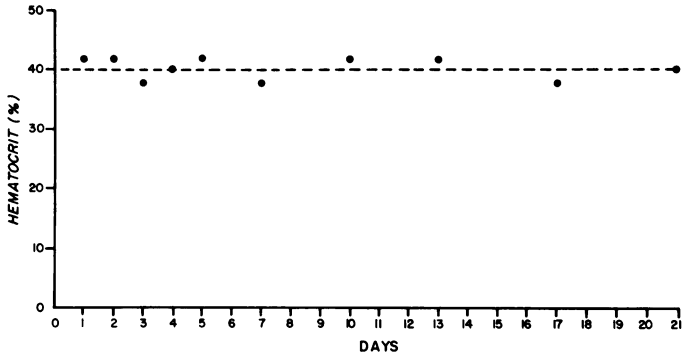

FIG. 1. Hematocrit through 21 days of storage.

ported by the relative paucity of ^{125}I fibrinogen on the screens during these first 10 days. It is likely that significant platelet function has disappeared by day 4. The continued response of platelet to ADP during this period is difficult to explain but suggests some residual ability of non-viable platelets to respond to ADP. Platelet viability is generally absent beyond 3 or 4 days of storage so that the observed response does not suggest normal platelet function beyond 4 days. The fact that no further microaggregates from beyond 4 days implies absence of platelet function and is consistent with published observations on platelet viability.[1,11] The exact nature of the observed response of platelets, stored beyond 4 days, to ADP remains obscure.

Studies with ^{125}I tagged fibrinogen indicate that until 10 days, fibrin contributes little to the microaggregate bulk. Beyond 10 days, however, SFP weight increases rapidly, paralleled by an increase in ^{125}I counts on the screen.

It, therefore, appears that microaggregates form in stored blood after 2 days of storage, primarily as a consequence of platelet aggregation. Furthermore, most of the matetrial is composed of platelets. From 4 to 10 days of storage, little change occurs in the amount of microaggregates in the blood. At about 10 days, however, fibrin begins to accumulate progressively on pre-existing platelet aggregates resulting in a progressive increase in the mass of microaggregates in the blood. This implies that the actual number of particles do not increase after the first 4 or 5 days of storage, but that further increase of microaggregate mass is due to deposition of fibrin on pre-existing platelet aggregates.

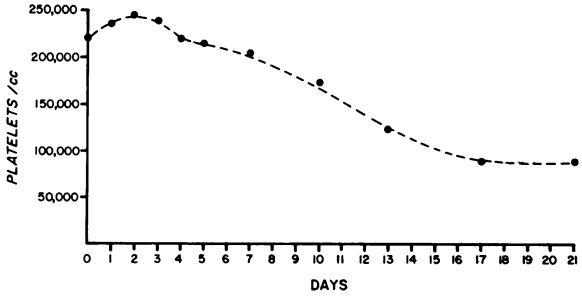

FIG. 2. Platelet count.

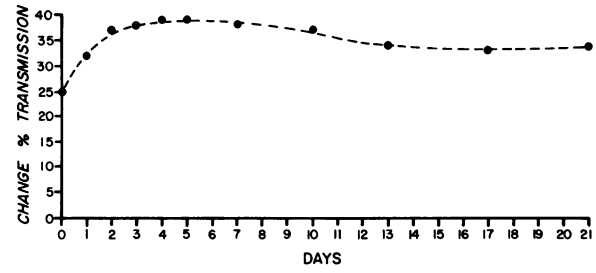

FIG. 3. Platelet aggregation response to ADP.

Microaggregates have been previously implicated in the pathogenesis of post-traumatic pulmonary insufficiency. It is not known whether platelet aggregates alone will produce pulmonary microemboli of any physiologic significance. It is conceivable that the risk of pulmonary damage is considerably greater with larger microemboli composed of both platelets and fibrin. If the latter is the case, then use of banked blood stored less than 10 days would circumvent the problem. On the other hand, if small microaggregate emboli composed primarily of platelets can produce pulmonary damage, then prevention of such early microaggregate formation and/or more effective filtration of all blood stored for over 2 days should be considered. Further investigation is currently underway to answer these questions.

Summary

Banked blood, collected in ACD solution and stored at 4° C., develops microaggregates which form during the second to the fifth day of storage. Microaggregate formation is accompanied by a transient increase in platelet aggregation to ADP and suggests that these are principally platelet aggregates. Microaggregate weight remains

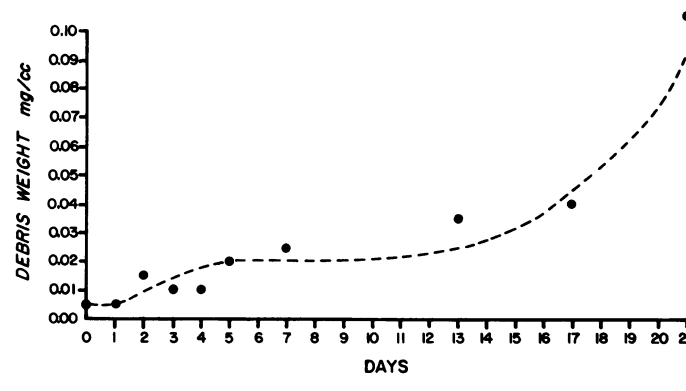

FIG. 4. Debris accumulation.

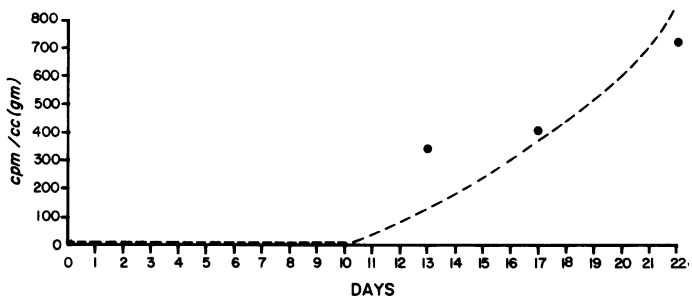

FIG. 5. ^{125}I fibrogen cpm/cc (gm) corrected to day 0 counts.

stable until 10 days beyond which a progressive increase is noted. Studies with ^{125}I tagged fibrinogen suggest that this increase beyond 10 days is due primarily to accumulation of fibrin on previously formed platelet aggregates.

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