SEVENTH ANNUAL REPORT

HUMAN PLATELET SENESCECE

ANNUAL SUMMARY REPORT

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Human Platelet Senescence

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Platelet senescence
Platelet differential centrifugation
Two dimensional polyacrylamide gel electrophoresis
Platelet subpopulations membranes
Enrichment of platelet collections with megathrombocytes

Evidence that megathrombocytes are released early from the bone marrow under basal conditions.

Evidence for a non-splenic as well as splenic platelet pool which is rapidly mobilizable during exercise and/or epinephrine administration.

Evidence that megathrombocyte number determines platelet function.
For the past year our laboratory has been engaged in 3 areas of platelet (and megathrombocyte) research with regard to production, sequestration, mobilization and function.

1. **Evidence that megathrombocytes are released early from the bone marrow under basal conditions.**

2. **Evidence for a non-splenic as well as splenic platelet pool which is rapidly mobilizable during exercise and/or epinephrine administration.**

3. **Evidence that megathrombocyte number determines platelet function.**

**SUMMARY OF PROJECTS**

1. **Evidence that megathrombocytes are released early from the bone marrow under basal conditions.**

Rabbits were treated with intravenous $^{75}$Se-selenomethionine, a cohort label which enters megakaryocytes but not peripheral blood platelets. Platelets released from the bone marrow however, do contain the isotope. With this technique, a cohort of young cells can be followed with time. Platelets were also separated into different density populations by centrifugation in their own platelet-rich plasma (anticoagulated with ACD-A) at 800 RPM, 1200 RPM, 1600 RPM, 1800 RPM and 2000 RPM in a Sorvall RC3 Centrifuge. The successive platelet pellets were suspended in an isotonic Ringer solution and sized on an electronic particle Coulter Counter P64 Channel Analyzer. In this fashion, a graded series of platelet populations could be isolated of decreasing density and volume since the lighter platelets had a relatively smaller platelet volume. The specific activity of each of these platelet populations was determined with respect to CPM $^{75}$Se-selenomethionine per platelet.

The results obtained (Figure 1) indicated that heavy-large platelets contained more isotope than lighter-smaller platelets on days 1 thru 7. On day 1, isotope was present in heavier-larger platelets (i.e. 800 RPM > 1200 RPM > 1600 RPM). On day 2, isotope was also present in 1800 RPM platelets and on day 3, isotope was also present in 2000 RPM platelets. Assuming homogenous distribution of isotope within a platelet, these data indicate that heavy-large platelets are released early from the bone marrow and suggest that they either become smaller and lighter with time or that small-light platelets are released from the marrow at a later time interval.

2a. **Preferential splenic sequestration of megathrombocytes.**

Rabbits were either splenectomized or subjected to splenic blockade with phenylhydrazine in order to determine the splenic platelet and megathrombocyte (large-platelet) pools. The average splenic platelet pool calculated from both methods was 35% of total platelets. The average splenic megathrombocyte pool was 54% of total megathrombocytes. Adrenaline injection into rabbits or dogs revealed a rapid increase in both platelet count and megathrombocyte number which peaked at 2-6 min and returned toward normal in 5-10 min. The platelet volume distribution curve was shifted to the right, indicating the release of large platelets (megathrombocytes) into the circulation. The peak rise in megathrombocyte number was significantly greater than the peak rise in platelet count. It is concluded that the spleen preferentially sequesters megathrombocytes.
2b. Evidence for a non-splenic platelet pool.

The spleen is the source of a rapidly-mobilizable pool of platelets representing 30-40% of the total population; and preferentially sequesters megathrombocytes. We have examined this platelet pool in intact and splenectomized rabbits, dogs and humans following epinephrine administration and/or exercise and provide evidence for an additional, non-splenic platelet pool.

A bolus of intravenous epinephrine was given to 9 intact rabbits (15 μg/kgm) and 6 dogs (11 μg/kgm). The platelet count and megathrombocyte number rose 1.32 and 2.74 fold respectively in rabbits and 1.48 and 1.88 fold in dogs peaking at 2 to 4 minutes and returned to basal levels at 8 to 12 minutes for both. Seven healthy adults were subjected to rigorous exercise (heart rate>150/min) for 11 minutes and raised their platelet count 1.48 fold and megathrombocyte number 2.53 fold peaking at 6 to 8 minutes and returning to basal levels at 10 to 15 minutes. Splenectomized animals and humans subjected to the same protocol gave the following results for platelet count and megathrombocyte number respectively: 8 rabbits, 1.15 and 1.40 fold; 4 dogs, 1.31 and 1.36 fold; 2 humans, 1.42 and 1.69 fold with similar peak intervals and return to basal level as with an intact spleen. Two dogs subjected to constant epinephrine infusion at 11μg/kgm/min for 12 minutes gave a similar peak rise and return to baseline of platelet count and megathrombocyte number. One dog subjected to a bolus of epinephrine followed by a second bolus 15 minutes later, gave a similar peak rise and return to baseline on both occasions. It is concluded that a rapidly-mobilizable, non-splenic platelet pool exists which is responsive to epinephrine and/or exercise. The peak response is followed by a refractory period of 12 to 15 minutes. This pool represents 13 to 30% of the total platelet population and is not preferentially enriched with megathrombocytes.

3. Evidence that megathrombocyte number determines platelet function.

Thirty-four healthy adults were examined for platelet count, platelet volume distribution (Coulter Counter, P64 Channel Analyzer) and platelet function (Bio/Data Aggregometer). The platelet count was inversely proportional to megathrombocyte index (MI), r = -0.56 (P<0.001). Maximum ADP or connective tissue velocity was determined from the average of two concentrations of either aggregating agent with undiluted PRP or PRP diluted 1:2. Sixteen of 32 patients gave higher velocities with diluted PRP, averaging 25% greater than undiluted samples. The maximum average ADP velocity correlated with MI, r = +0.62 (P<0.001), whereas it did not correlate with the median platelet volume, r = +0.27 (P>0.1) or the mode platelet volume, r = +0.22 (P>0.1). The maximum average connective tissue velocity correlated with MI, r = +0.59 (P<0.001), but correlated poorly with the mode platelet volume, r = +0.39 (P<0.05) or the median platelet volume, r = +0.40 (P<0.05). Platelet volume distribution curves of residual non-aggregated platelets revealed relative absence of larger platelets. 

Conclusions: 1) Platelet count is inversely proportional to MI. 2) Platelet aggregation velocity correlates with megathrombocyte number, not with median or mode platelet volume, suggesting that megathrombocyte number is critical for platelet function.
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