POSTER SYMPOSIUM XIII

Platelets: Immunology.

PLATELET ALTERATIONS CAUSED BY ANTIPLATELET ANTIBODIES IN THE CIRCULATION. T. Nagasawa, B.K. Kim and M.G. Baldini. The Memorial Hospital, Pawtucket and Brown University, Providence, Rhode Island, U.S.A.

It is known that antiplatelet antibodies cause loss of platelet cytoplasmic and granular contents in vitro. It is, however, unknown whether similar platelet changes occur in vivo, in the circulation, leading to destruction and phagocytosis of platelets in the R.E. system. To study this possibility a rabbit model was devised. Severe and stable thrombocytopenia was first produced in rabbits by one intravenous injection of Adriamycin. Large numbers of allogenic platelets labeled in vitro with ^{51}Cr and ^{14}C -serotonin were then infused to raise the circulating platelet count to $180\text{--}250 \times 10^3/\text{mm}^3$. A dilute heteroimmune antiplatelet serum prepared in the guinea pig was infused intravenously and platelet samples were collected four times during the subsequent 30 minutes to 24 hours. Platelet hexokinase and β -glucuronidase, ^{14}C -serotonin and ^{51}Cr were measured. Within the first 60 min the specific activity of ^{51}Cr in platelets decreased by 21%, ^{14}C -serotonin declined by 30%, hexokinase by 5% and β -glucuronidase by 29%. During the subsequent 24 hours only ^{51}Cr and hexokinase registered a mild decrease but ^{14}C -serotonin and β -glucuronidase remained essentially unchanged. In a second series of experiments the effect of platelet alloantibodies was studied in rabbits previously immunized with allogenic platelets. The decline in the specific activities of the enzymes and ^{14}C -serotonin was similar to that observed in animals treated with heteroimmune sera but loss of ^{51}Cr was more severe. These results demonstrate that the platelets remaining in the circulation after the disappearance of the immediate effect of hetero- or alloantibodies were qualitatively altered with a severe reduction of their granular and cytoplasmic contents.

AN IMMUNOENZYME HISTOCHEMICAL TECHNIQUE FOR DETECTING PLATELET ANTIBODIES FROM THE SERUM OF CHILDREN WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP). D. Tate, R. Sorenson, J.M. Gerrard, W. Krivit and J.G. White. Departments of Anatomy and Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota U.S.A.

A simple reproducible, semiquantitative immunohistochemical assay for the detection of antiplatelet antibodies has been developed and is adaptable for clinical use. Blood smears from normal donors are incubated with test sera and platelet bound immunoglobulins are detected using goat anti-human immunoglobulin G₁A₁M₁ conjugated to horseradish peroxidase. The blood smears are observed under phase contrast microscopy and the labelling of peroxidase on individual human platelets is quantitated, by comparison with standards graded 0-4. For each test sera a hundred platelets are examined. Control platelets showed indices which ranged from 0.34 to 1.06 (mean 0.67 ± 0.06 SE, $n = 12$). Sera samples from ten patients with active ITP showed significantly increased antiplatelet antibody levels with indices ranging from 1.47 to 3.04 (mean 1.99 ± 0.18 SE). Seven children with thrombocytopenia due to other causes were in the same range as normal children (mean 0.62 ± 0.07 SE). Five patients who had had multiple platelet transfusions did have significantly elevated levels of antiplatelet antibodies (mean 1.16 ± 0.12 SE). However, when these individuals were evaluated using blood smears from HLA matched donors levels of antiplatelet antibody decreased to control levels (mean 0.46 ± 0.08 SE). The results suggest that this test may be useful for the evaluation of children with ITP and can differentiate between autoantibodies due to ITP and isoantibodies resulting from multiple platelet transfusions.