Platelet Satellitism

By Carl R. Keldsberg and John Swanson

Platelet adherence to polymorphonuclear leukocytes, or so-called platelet satellitism, has, to our knowledge, been reported in only four patients. We had the opportunity to study this phenomenon in two patients. Platelet satellitism was only seen in EDTA anticoagulated blood, and the platelets were seen to surround polymorphonuclear leukocytes only. Electron microscopic studies demonstrated focally apposed regions of platelet and neutrophil plasma membranes. Phagocytosis of platelets was also observed. In vivo and in vitro platelet functions were normal. Platelet satellitism is an in vitro phenomenon, the cause of which is unknown. We are unable to relate it to functional abnormalities of the blood, the clinical condition of the patient, or to drugs. This phenomenon has some clinical importance in that it causes spurious thrombocytopenia.

Adherence of platelets to polymorphonuclear leukocytes ("platelet satellitism") has been described in a few brief reports and is thought to be relatively rare. In a single week we encountered two patients who exhibited spurious thrombocytopenia which was attributable to platelet satellitism. This phenomenon was observed only in blood anticoagulated with ethylenediamine tetraacetate (EDTA) and was characterized by seeming association of platelets with polymorphonuclear leukocytes but not with eosinophils, basophils, lymphocytes, or monocytes. Studies were carried out in an attempt to more fully document this phenomenon and elucidate the ultrastructural details of the platelet-polymorphonuclear leukocyte relationships.

MATERIALS AND METHODS

Standard hematologic methods were performed as described by Cartwright. The platelet count was done with an electronic counter (Coulter Fn, Coulter Electronics, Inc., Hialeah, Fla.) using a modification of Fry and Hoak's centrifugation method. All blood samples were drawn in plastic syringes. Platelet adhesiveness was measured by the method of Hellem, and Borchgrevink's bleeding time was used. Platelet aggregation was measured using a Chrono-Log Aggregometer (Chrono-Log Corp., Broomall, Pa.).

To study the effect of the patient's plasma, platelets, and white blood cells on normal platelets and white blood cells, the following studies were done: (1) patient's EDTA plasma was mixed with normal platelets and normal white blood cells, (2) patient's platelets were mixed with normal white blood cells, and (3) patient's white blood cells were mixed with normal platelets. Platelet-rich plasma was obtained by centrifuging EDTA anticoagulated whole blood for 4 min at 1000 g. Platelet buttons for concentrated platelet suspensions were made by centrifuging platelet-rich plasma for 10 min at 2000 g. Resuspensions to the desired concentrations were made in ABO type specific plasma. Separation of leukocytes from blood was made according to the method of Boyum.

From the Division of Clinical Pathology and the Department of Pathology, University of Utah Medical Center, Salt Lake City, Utah 84132.

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Carl R. Kjeldsberg, M.D.: Assistant Professor of Pathology, Division of Clinical Pathology, University of Utah Medical Center, Salt Lake City, Utah 84132. John Swanson, M.D.: Associate Professor of Pathology, Department of Pathology, University of Utah Medical Center, Salt Lake City, Utah, 84132.

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Electron Microscopic Procedures

Leukocyte platelet associations were studied by electron microscopy of thin-sectioned material. Preparation of specimens for thin sectioning included partially purifying the leukocytes and thrombocytes by mixing EDTA containing blood with an equal amount of 2% gelatin in 0.9% sodium chloride and allowing erythrocytic elements to sediment during incubation for 20 min at 37°C. After centrifugation of plasma containing both leukocytes and platelets (500 rpm for 5 min), residual erythrocytes were lysed by addition of 0.9% ammonium chloride (5 min, 20°C), and the pelleted leukocyte-platelet mixture was washed with Earle’s basic salt solution. Following resedimentation, the washed cells were fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate and processed as previously described. Electron microscopic examinations were carried out with either a Zeiss 9 (Carl Zeiss, Inc.) or an Elmiskop 1A (Siemens Corp.) microscope.

CASE REPORTS

Case 1.
A 47-yr-old white woman was admitted to the University of Utah Medical Center because of a left leg fracture. Previous clinical history was noncontributory. The physical examination was within normal limits except for a fractured left femur. The patient had not been taking any medication for the last 4 wk, and there was no history of alcohol abuse. The hemoglobin was 14 g/100 ml, and the red blood cell indices were within normal limits. The white blood count was 6200/cu mm with a normal differential count. The platelet count was 46,000/cu mm. Routine laboratory tests, including serum protein electrophoresis and serum immunoelectrophoresis, were all normal. A blood smear made from EDTA anticoagulated blood showed the majority of the platelets to adhere to polymorphonuclear leukocytes (Fig. 1A).

Case 2.
A 50-yr-old white man was admitted to Veterans Administration Hospital, Salt Lake City, Utah, because of acute withdrawal from alcohol intoxication. The patient had not been taking any other drugs or medication. Precious clinical history revealed chronic alcoholism with cirrhosis of the liver and a duodenal ulcer. The physical examination revealed a slightly enlarged liver but was otherwise normal. The blood count showed hypochromic, microcytic anemia with a hemoglobin of 11.5 g/100 ml. The white blood count was 10,500/cu mm with a normal differential count, and the platelet count was 52,000/cu mm. A blood smear made from EDTA anticoagulated blood showed many polymorphonuclear leukocytes to be surrounded by platelets, most of which were apparently adherent to the leukocytes. The serum protein electrophoresis and serum immunoelectrophoresis were normal. Other laboratory tests showed mild liver dysfunction.

RESULTS
The phenomenon of platelet adherence to leukocytes, or so-called platelet satellitism, was seen in both patients in multiple blood smears made during their hospital stay. The platelets were seen to adhere to, and in some instances appeared to be engulfed by, polymorphonuclear leukocytes. Platelet satellites were not seen around lymphocytes, monocytes, eosinophils, or basophils (Fig. 1B and C). The platelets appeared to have a normal morphology with light microscopy. Platelet satellitism was seen in fresh and several-hours-old blood samples, and it was seen in all areas of the blood smear. Wet, unstained blood smears made from EDTA anticoagulated blood revealed similar features to those that were fixed and stained (Fig. 2). Varying the temperature of the blood sample from 4°C to 37°C had no apparent affect on the ability of the platelets to adhere to the leukocytes.

The phenomenon of platelet satellitism was only seen in blood smears made from EDTA anticoagulated blood. When double oxalate, sodium citrate, or
Platelet satellitism around polymorphonuclear leukocytes is shown in a wet, unstained preparation using interference contrast microscopy. EDTA anticoagulated blood x 560.

Fig. 1. (A) Blood smear made from EDTA anticoagulated blood showing platelet satellitism around polymorphonuclear leukocytes. Wright stain x 1400. (B) Blood smear made from EDTA anticoagulated blood shows platelet satellitism around polymorphonuclear leukocytes but not around a lymphocyte. Wright stain x 1400. (C) Blood smear made from EDTA anticoagulated blood fails to show satellitism around a monocyte. Wright stain x 1400.

Fig. 2. Platelet satellitism around polymorphonuclear leukocytes is shown in a wet, unstained preparation using interference contrast microscopy. EDTA anticoagulated blood x 560.
heparin was used as an anticoagulant, or if no anticoagulant was used, satellitism was not seen. Platelet adherence to leukocytes was not found in blood smears from normal individuals when the patient’s plasma, platelets, and white blood cells were mixed with platelets and white blood cells or platelets, respectively, obtained from a normal individual.

Phase microscopy of EDTA anticoagulated blood from both patients showed the majority of the platelets adherent to leukocytes, and an accurate platelet count could not be obtained. With EDTA anticoagulated blood, the electronic platelet counts were 46,000/cu mm and 52,000/cu mm, respectively, in the two patients described. When oxalate was used instead of EDTA as an anticoagulant, the platelet counts in the same two patients were 320,000/cu mm and 360,000/cu mm, respectively. In vitro platelet function studies, including the bleeding time, platelet adhesion, and platelet aggregation, were normal in both patients. In vivo platelet function also appeared normal in that the first patient had surgical pinning of the fractured hip without any excessive bleeding.

Most of the polymorphonuclear leukocytes observed by electron microscopy in thin sections were surrounded by one or more platelets, and the types of associations between these two cell types varied. In a few instances, platelets appeared to be partially (Fig. 3A) or completely enclosed within phagocytic vacuoles of a neutrophil. In the majority of instances, however, platelet-neutrophil associations seemed to consist of focal areas of contact between the limiting plasma membranes of the two cell types, as shown in Figs. 3B–E. Contact between the cells appeared to be mediated across an electron-lucent space separating the external-most aspects of the apposing plasma membranes. In some instances, contact occurred via a pseudopod from the neutrophil (Fig. 3B), and in others contact involved a short process from the platelets (Fig. 3C–E).

DISCUSSION

The phenomenon of platelet satellitism was seen by light microscopy only in blood smears made from EDTA anticoagulated blood. It did not occur in smears made from nonanticoagulated blood or if double oxalate, sodium citrate, or heparin were employed. The platelets were seen to surround and adhere to polymorphonuclear leukocytes, and sometimes they appeared to be engulfed by the leukocytes. Platelet adherence to lymphocytes, monocytes, eosinophils, or basophils was not seen. The morphology of the individual platelets and leukocytes appeared normal by light microscopy.

Platelet satellitism studied by electron microscopic techniques corroborates the light microscopic observations and demonstrates several morphologic forms of contact between neutrophils and platelets. Focal apposition of the plasma membranes of the two cell types with an interposed electron-lucent space is the most frequently observed form of contact. In some instances phagocytosis of a platelet by a neutrophil was apparent. The ultrastructural studies do not define particular characteristics of the apposed plasma membranes of platelets or neutrophils that might be related to the phenomenon of satellitism.

We were unable to transfer platelet-leukocyte satellitism to normal blood. Neither the patient’s plasma, platelets, and/or white blood cells, when mixed
Fig. 3. In thin sections of neutrophils (N) and platelets (P), numerous forms of contact between the two cell types are observed. Apposition of the cells (arrows) involves focal segments of their plasmalemmata. In a few instances such focal apposition is accompanied by partial enclosure of the platelet within a concavity of the neutrophil, as in (A). A pseudopod of the neutrophil (B) may participate in the association with a platelet, but usually a rather blunt, rounded protuberance from the platelet seems to mediate the association with the adjacent neutrophil (C, D, E). Magnifications: 3A and 3B x 25,000; 3C x 11,500; 3D and 3E (enlarged portions of 3C) x 44,000. Lead citrate and uranyl acetate stained.
with normal white blood cells and/or normal platelets, produced any platelet-leukocyte interactions. Platelet functions as measured by bleeding time, platelet aggregation, and platelet adhesion were all within normal limits.

Platelet satellitism is apparently an in vitro phenomenon, the cause of which is unknown. It cannot be attributed directly to EDTA, since we have seen thousands of peripheral blood smears made from EDTA anticoagulated blood which do not exhibit platelet satellitism. The phenomenon is not a function of the peripheral blood smearing method, since satellitism was also seen in wet, unstained preparations of EDTA anticoagulated blood. Varying the temperature of the blood sample from 4°C to 37°C had no apparent affect on the ability of the platelets to adhere to leukocytes. In addition, we are unable to relate it to functional abnormalities of the blood, the clinical condition of the patient, nor to drugs.

To our knowledge, platelet satellitism has previously been described in only four patients. A mention of this as a cause of spurious thrombocytopenia has not been made, however. McDonald, Dodds, and Cruickshank described platelet satellitism in association with thrombocythemia. Platelet satellitism has also been noted in Bechet’s disease, apparently only when the disease is active.

Platelet satellitism has some clinical importance in that it causes spurious thrombocytopenia. This was seen in both of our patients. Satellitism was, in fact, discovered in one of the patients because the platelet count was inexplicably low. The phenomenon was temporary in that it was not seen in either patient 3 wk following discharge from the hospital.

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