Platelet Satellitosis: A Case Report with Laboratory Characterization and Quantitative Evaluation*

GENE L. GULATI, Ph.D., BONG H. HYUN, M.D., D.Sc.,†
and MARY WOODALL, B.S., M.T.(ASCP)SH

Department of Pathology, Muhlenberg Hospital,
Plainfield, NJ 07061
and
Rutgers Medical School,
Piscataway, NJ 08854

ABSTRACT

In an attempt to understand better the phenomenon of platelet satellitosis (PS) and its pathogenesis and clinical relevance, in vitro and in vivo studies were performed. Quantitative evaluation of PS was carried out by (1) determining the combined percentage of neutrophils and bands showing PS and (2) calculating the PS score in a manner similar to the leukocyte alkaline phosphatase score. Among ethylenediamine tetraacetic acid (EDTA)-treated, heparinized, citrated and non-anticoagulated blood samples, only films made from EDTA-treated blood revealed PS. By light microscopy, PS was characterized by adherence of platelets to neutrophils and bands. Examination of films prepared at timed intervals from EDTA-treated blood kept at room temperature (RT) showed maximal PS at one hour. A significant decline in PS ensued after the blood had stood at RT for four hours or longer. Day-to-day variations in the degree of PS followed a cyclic pattern with a cycle length of approximately 20 days, but they did not correlate with the patient’s clinical status or any other laboratory findings. Results of in vitro studies suggested that the patient’s plasma contained a substance which would induce platelet satellitosis in normal EDTA anticoagulated blood and in buffy coats harvested from the patient’s citrated blood.

Over the past 25 years the phenomenon of platelet adherence to leukocytes, generally referred to as platelet satellitosis (PS) or platelet satellitism, has been described in a small but clinically diverse group of patients.1-16 The exact nature of this phenomenon, its pathophysiology and clinical significance are not well understood. Recently PS was observed in the blood film of a hospitalized patient while the present authors performed the routine differential leukocyte count. In
vivo and in vitro studies carried out on this patient in an attempt to investigate the nature and the pathogenesis of PS are reported in this communication. Procedures developed and utilized to evaluate PS quantitatively are also detailed.

Case Report

A 67 year old, obese, white female was discharged from the hospital following treatment for cerebrovascular insufficiency, hypertension, diabetes mellitus with Kimmelstiel-Wilson syndrome, hypothyroidism and genitourinary tract infection (E. coli). She was readmitted one month later because of an episode of syncope followed by weakness.

Physical examination revealed psoriatic lesions on the legs. Initial important laboratory findings were as follows: hemoglobin 11.0 g per dl (1.71 mmol per l), total leukocyte count 12,000 per µl (12 x 10⁹ per l), with a differential count of 46 percent neutrophils, 18 percent bands, 21 percent lymphocytes, 4 percent monocytes, 8 percent eosinophils, 2 percent basophils and 1 percent metamyelocytes. Platelet satellitosis was noted on peripheral blood films. A platelet count performed one week after admission was 325,000 per µl (0.325 x 10¹² per l). The blood glucose was 150 mg per dl (8.25 mmol per 1), urea nitrogen 26 mg per dl (9.3 mmol per 1), uric acid 6.6 mg per dl (0.39 mmol per 1), serum albumin 2.5 g per dl (0.39 mmol per 1), alkaline phosphatase 232 IU per 1, lactate dehydrogenase 240 IU per l, and glutamic oxaloacetic transaminase (SGOT) 85 IU per l. Urinalysis revealed more than 100 white cells per high-power field, moderate protein and many bacteria.

During the course of hospitalization (10 weeks) the patient received a total of 26 drugs including diuretics, ataractics, analgesics, cardiotonics, anticoagulants, hormonal preparations, antibiotics, anti-hypertensive agents and insulin. One month after admission she developed gangrene of one foot requiring amputation. Subsequently the patient deteriorated, and died on the 76th hospital day. The major findings at autopsy included cerebral atherosclerosis with marked stenosis of the right internal carotid artery, acute infarction of the right cerebral hemisphere and cholangiocarcinoma with metastases to para-aortic and peripancreatic lymph nodes.

Methods and Materials

QUANTITATIVE EVALUATION OF PLATELET SATELLITOSIS

Peripheral blood (PB) films prepared by the conventional slide method and stained with Wright stain were evaluated for PS quantitatively by (1) determining the combined percentage of neutrophils and bands showing platelet satellitosis (%CPS) and (2) calculating the PS score in a manner similar to the leukocyte alkaline phosphatase score. As shown in table I, a scale of 0 (zero) to 4+ was used to score cells.

On initial screening of some PB films, it was noted that the PS-positive cells (neutrophils and bands), though seen in both thick and thin areas of the film, were present in relatively larger numbers in the thick area. Therefore, a total of 400 neutrophils and bands combined (200 each from the thin and thick areas) were counted to obtain the %CPS and PS score per 100 cells.

IN VIVO STUDY

During the course of 10 weeks of hospitalization, the patient's peripheral blood films were studied and prepared routinely 19 times as part of the CBC. Each film was evaluated for %CPS and the PS score. These were then plotted for the entire 10 week period on linear graph paper. This study permitted assessment of variations in the degree of PS under in vivo circumstances and of the relation with the clinical course and the pertinent laboratory findings.

IN VITRO STUDIES

Study 1: Blood samples were collected from the patient by direct finger stick in ethylenediamine tetraacetic acid (EDTA), in sodium citrate and in heparin. Films were prepared by the conventional slide method within 15 minutes of sample preparation.
collection, stained with Wright stain and evaluated for PS.

**Study 2:** Patient's EDTA-treated blood was allowed to stand at room temperature (RT) up to a maximum of 28 hours. Films prepared from this sample at specified intervals (15 minutes, 1, 2, 3, 4, 12 and 28 hours) were examined for PS after staining with Wright stain.

**Study 3:** EDTA-anticoagulated whole blood from an ABO-compatible healthy subject and buffy coats isolated from patient's citrated blood were incubated separately after mixing with either platelet-rich plasma (PRP) or platelet-poor plasma (PPP) harvested from patient's EDTA-treated blood. Incubations were carried out at room temperature and at 37°C, some for 30 minutes and others for two hours. Films prepared from these incubated mixtures were stained with Wright stain and examined for PS.

**Results**

**In Vivo Study**

**Variations in the Degree of PS During Hospital Course.** The degree of PS as measured by % CPS and the PS score followed a cyclic pattern with a cycle length of approximately 20 days (figure 1). Of the total 19 PB films examined over the 70 day period, one showed marked PS (%CPS = 88, PS score = 268), 10 moderate degree of PS (%CPS = 20 to 50, PS score = 50 to 108), 4 mild (%CPS = 8 to 13, PS score = 10 to 21), and the remaining 4 revealed either an insignificant degree of PS (%CPS = 2 to 3, PS score = 3 to 4) or none at all. The PB films with moderate to marked degrees of PS also revealed a few aggregates of PS-positive cells (figure 2). The PS score always paralleled the %CPS (figure 1).

**In Vitro Studies**

**Anticoagulants and the Phenomenon of PS.** Among the films prepared from non-anticoagulated, EDTA-treated, citrated and heparinized blood samples, only those prepared from EDTA-treated blood were found to be positive for PS. These involved mainly neutrophils with a small number of bands but none of the other leukocytes (lymphocytes, monocytes, eosinophils, basophils, myelocytes and metamyelocytes).

**Variations in the Degree of PS with Time In Vitro.** Results of this study are illustrated in figure 3. A film prepared from EDTA-treated blood at 15 minutes from the time of collection revealed a
Figure 2. Peripheral blood film showing marked degree of platelet satellitosis and the tendency of neutrophils with platelet satellitosis to aggregate (Wright stain, × 250).

%CPS value of 45 and a PS score of 84. By one hour the degree of PS had reached the peak level with a %CPS of 78 and a PS score of 188. This level of PS was maintained for the subsequent two hours. It then appeared to decline, with the film prepared at four hours showing a %CPS of 60 and PS score of 143. Poor cell morphology encountered in films prepared at 12 and 28 hour intervals made it difficult to analyze the degree of PS, but examination of these films gave the impression that the degree of PS had significantly and progressively declined.

In-Vitro Induction of PS. A significant degree of PS (%CPS of 10 or higher, PS

Figure 3. Changes in the degree of platelet satellitosis revealed by the examination of films prepared at serial intervals from the patient’s EDTA-treated blood sample following incubation at room temperature.

- Percentage of cells (neutrophils and bands) showing platelet satellitosis (%CPS)
- Platelet satellitosis score (PS score).

Values at these points are rough estimates, made necessary by poor cell morphology.
score of 14 or higher) was observed in films prepared from room temperature incubated mixtures containing patients’ EDTA plasma (PRP or PPP) and either patient’s citrated buffy coat or normal EDTA-anticoagulated whole blood. PS was not seen in films prepared from similar mixtures incubated at 37°C.

Discussion

Our findings that (1) only EDTA-treated blood revealed PS and (2) the phenomenon of PS involved primarily neutrophils with small number of bands but none of the other leukocytes are essentially in accord with the earlier reports of Crome and Barkhan, Kjeldsberg, Kjeldsberg and Swanson, Prchal and Blakely, and Signy and Green. The findings do not agree with Field and MacLeod, who reportedly found PS also in films of citrated, oxalated and heparinized blood, or Ravel and Bassart, who observed PS in films prepared from nonanticoagulated blood in addition to those from EDTA-treated blood. Thus, the concept proposed by Field and MacLeod that PS is probably an \textit{in vitro} phenomenon is supported by several but not all of the reports in the literature, particularly not by those indicating a parallel course between the phenomenon of PS and the clinical condition of the patient.

That PS is a transient phenomenon is suggested by the observation that incubation of EDTA-treated blood at RT for four hours or longer resulted in significant decline in both the %CPS and the PS score. A marked increase in the degree of PS seen during the initial hour of incubation of the EDTA-treated blood at RT necessitated the preparation of films consistently at a preset interval of preferably one to three hours from the time of blood collection, for the study of day-to-day variations in the degree of PS. Results of the study carried out to monitor such variations, utilizing PB films prepared usually one to two hours after blood collection, revealed a cyclic pattern in %CPS and the PS score with the cycle length being approximately 20 days. A similar pattern of about the same cycle length has been previously described by Morley for blood neutrophil concentration in healthy men.

Whether or not these two phenomena are related in any way is not known. Variations in %CPS and the PS score did not correlate with the status of the patient or abnormal hematologic findings such as leukocytosis, neutrophilia, eosinophilia, shift to the left, or apparent thrombocytosis, as judged by scan of PB films. Additional abnormal laboratory findings the patient exhibited were elevated levels for blood glucose, urea nitrogen, alkaline phosphatase, \textit{glutamic oxaloacetic transaminase} as well as triglycerides (type IV hyperlipoproteinemia) and low levels of serum albumin with normal total protein content. These determinations and platelet counts were not performed frequently enough to be of value in establishing a relation between any of these findings and the phenomenon of PS.

Attempts to relate the phenomenon of PS to the effect of a specific drug or group of drugs have also been reported as unsuccessful. In our patient, such attempts were hindered by the fact that her complete therapeutic regimen consisted of a long list of drugs (26 total).

Results of our \textit{in vitro} Study 3 indicate that PRP and PPP prepared from the patient’s EDTA-treated blood were capable of inducing PS in normal EDTA-treated whole blood and in buffy coats harvested from the citrated blood of the patient at RT but not at 37°C. In similar studies carried out by Bolton and Boyd, it was found that cell-free EDTA-plasma from their patient (known to exhibit PS) induced PS in normal EDTA-treated blood to a greater degree at 4°C than at RT or at 37°C. Transfer of PS activity to normal whole blood by the plasma of patients known to exhibit PS has also been reported by
McGregor and associates.\textsuperscript{10} Recently, Greipp and Gralnick have successfully demonstrated transfer of PS activity to normal whole blood and buffy coat using serum at 37°C and 25°C.\textsuperscript{5} These reports and ours suggest that the plasma and/or serum of such patients contains a factor (we prefer to call it PS factor, PSF) which is capable of inducing PS \textit{in vitro}.

Studies designed to investigate the nature of PSF have been few and have yielded variable results.\textsuperscript{2,5,10} PSF has been reported to be an immunoglobulin (IgG) by Greipp and Gralnick\textsuperscript{5} and a cryoprotein composed partly of cryofibrinogen by McGregor et al.\textsuperscript{10} From our own limited experimental data, it can only be hypothesized that the phenomenon of PS is mediated by PSF, which is probably a slow-acting humoral factor present in the plasma, that it somehow requires EDTA for its effect under \textit{in vitro} conditions and that it is reactive at RT. That RT is probably the optimum temperature for demonstrating PS activity \textit{in vitro} is also supported by studies\textsuperscript{12,15} in which investigators found a significantly lower degree of PS or none at all in EDTA-treated blood at 4°C, 28°C, 33°C and/or 37°C than at 20°C or 23°C. Further studies are necessary to determine the source, physicochemical nature and mechanism(s) of action of PSF.

Other important findings pertaining to PS and deserving mention here are that (1) PS causes spurious thrombocytopenia \textit{in vitro} when the counts are performed with electronic particle counters,\textsuperscript{2} (2) PS may be an antibody-mediated phenomenon and could be associated with thrombocytopenia \textit{in vivo}\textsuperscript{\textsuperscript{5}} and (3) clusters of PS-positive neutrophils are falsely interpreted as single large leukocytes with high peroxidase activity representing immature or possibly abnormal neutrophils by the Technicon Hemalog D.\textsuperscript{8} The infrequent platelet counts on our patient's blood were performed with a phase microscope soon after collection of the blood specimens, thereby eliminating the possibility of encountering spurious thrombocytopenia.

Our attempts to correlate the oscillations in the degree of PS with variations in the patient's condition have met with unrewarding results, as have similar attempts by many other investigators.\textsuperscript{2,4,7} Nevertheless, Prchal and Blakely in 1973 described a patient with long-standing Behçet's disease, in whom the degree of PS paralleled the clinical course.\textsuperscript{12} More recently Greipp and Gralnick have described two patients in whom the development of thrombocytopenia and pulmonary distress could be directly related to the phenomenon of PS.\textsuperscript{5} The variety of clinical conditions represented by the patients reported to have shown PS\textsuperscript{1,2,3,5,6,7,8,9,12,13,15,16} is too large even to attempt to establish a relation between the phenomenon of PS and any one or more clinical diagnoses. It may be noted, however, that a significant number of these patients (including ours) suffered from thrombosis or "thrombotic tendency" prior to, during and/or following the period PS was observed in their blood films. A cause-effect relation between the thrombotic problems and the PS remains to be established.

References


