Thrombocytopenia with a Circulating Platelet Agglutinin, Platelet Lysin and Clot Retraction Inhibitor

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INTEREST in the mechanism of development of thrombocytopenia has been greatly stimulated in recent years by reports of in vitro demonstrations of platelet agglutinins in the serum of a substantial proportion of patients with thrombocytopenia of obscure origin.1,2 Although thrombocytopenia in patients with cancer is usually the result of bone marrow invasion by the tumor, agglutinins have been reported in the serum of occasional cancer patients.1,3,4 The case reported in this paper describes a patient with cancer who developed sudden thrombocytopenia while under observation and in whose serum a platelet agglutinin was evident. In addition, the patient’s serum contained a platelet lysin and an inhibitor of normal clot retraction. The technics used to demonstrate platelet lysis and inhibition of clot retraction are described. The results of the study of sera from other patients with platelet agglutinins are included.

Case Report

J. P., a 56-year-old white female, was admitted to Memorial Center in August, 1956 complaining of malaise and fever of two months’ duration.

Present illness: Nine months before admission the patient noted a lump in her left breast. A diagnosis of carcinoma of the breast with supraclavicular and axillary lymph node metastases was made. Operation was advised but she refused. No biopsy was performed. During the next few months she received extensive x-ray therapy to the left breast, supraclavicular and axillary regions. The masses regressed, but three months before admission the one in the breast recurred. This was again treated with x-ray with prompt regression.

About two months before admission the patient began to feel weak, and a fever, which on occasion reached 104 F., was noted. Her appetite diminished and she lost 15 pounds in weight. She also began to complain of dyspnea on exertion. Except for aspirin, she received no medication.

Family history: The patient’s mother died at 91 of metastatic breast cancer. One sister died of cancer of the lung and one brother had a gastric resection for cancer of the stomach.

Past history: The patient had had three pregnancies and there were two living children. The third pregnancy, 20 years before admission, resulted in a miscarriage. She had had a hysterectomy in 1950 after five to six months of vaginal bleeding, presumably due to uterine fibroids. As ascertained from the patient and her family, she had never received a blood transfusion. There was no history of allergic or hemorrhagic disorders.

Physical examination: Her temperature was 100.5 F. There were postirradiation skin changes over the left breast, axilla and supraclavicular area. There was a slightly enlarged node in the left supraclavicular area. There were no other significant findings.

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Laboratory: Her hemoglobin was 10.0 Gm.%. The white blood cell count was 6,500/cu.mm. and the differential count was normal. The platelet count was 192,000/cu.mm. Routine urinalysis was negative. Fasting blood sugar was 114 mg.% and a glucose tolerance test showed a diabetic curve with a peak of 309 mg.% at 2 hours. Blood urea nitrogen was 12.1 mg.% and 24-hour urinary calcium determinations during periods of low calcium intake showed slight but definite elevations (183 to 245 mg. per 24 hours). Serum alkaline phosphatase activity was 4.0 Bodansky units.

Chest and skeletal x-rays were negative.

Attempted aspiration of the left supraclavicular node yielded only blood and fat.

Hospital course (Fig. 1): During the period of initial study in the hospital, the patient ran an irregular fever which, on occasion, reached as high as 104 F. but was usually around 100 F. On the 17th hospital day the patient received a blood transfusion. After about 250 cc. had run in, she had a severe reaction with a chill and sharp elevation of fever. The transfusion was discontinued and the patient promptly recovered. Investigation revealed no red cell incompatibility.

A platelet count on the 20th day was 184,000 cu.mm. In addition, occasional nucleated red blood cells were seen on the blood smear.

On the 22nd day, extensive petechiae were found on the face, neck and upper torso. There was considerable bleeding from the gums and a small hematoma appeared on the lateral aspect of the tongue. The tourniquet test was strongly positive. No platelets were seen on the following day. The hemoglobin was 10.1 Gm.%; the white cell count 7,300/cu.mm., with moderate shift to the left in the differential count; the reticulocyte count was 2.1%. The bleeding time was over 11 minutes. The clotting and prothrombin times were normal, but prothrombin consumption was poor. The direct and indirect Coombs' tests were negative.

On the 23rd day a second blood transfusion was given. As on the previous occasion, the patient had a severe chill and elevation of temperature after 200 ml. had run in, and again tests for red cell incompatibility and for hemolysis were negative.

Platelet counts were performed on several occasions between the 22nd and 38th days using white blood cell pipets according to the method of Brecher and Cronkite. The counts were consistently less than 700 cu.mm. and the few platelets seen did not appear normal.
A bone marrow aspiration was performed on the 28th day and showed a cellular
marrow with normoblastic hyperplasia. Megakaryocytes were plentiful and normal in form
except that no "platelet budding" could be seen. In addition numerous clumps of tumor
cells were present.

Between the 20th and 22nd hospital days, when the thrombocytopenia developed,
the only drugs the patient was receiving were Demerol, aspirin, and Equanil. All medi-
cations except Demerol were discontinued. Benadryl and Pyribenzamine were administered
after the transfusion reaction on the 23rd day.

Cortisone was begun on the 23rd day in a dose of 100 mg. per day. On the 25th day
500 ml. of fresh whole blood were transfused; a mild reaction occurred at the end of the
transfusion. The same type of reaction was observed five days later following the admin-
istration of the platelets concentrated from one unit of fresh blood. No increase in
platelet count occurred as a result of this transfusion. Because of the persistent thrombo-
cytopenia and hemorrhagic manifestations, on the 32nd day the dose of cortisone was
increased to 200 mg. per day, and on the 34th day this was supplemented by intramus-
cular ACTH gel in a dose of 120 units twice a day. On the 36th day the patient received
500 cc. of whole blood with no untoward reaction. The platelet count was still below
700 cu. mm. and oozing from the nose and mouth continued.

On the following day (37th day) the cortisone and ACTH were stopped and prednisone
was begun in a dose of 100 mg. per day. Five days later the platelet count began to
rise and reached normal levels by the 48th day. The hemorrhagic manifestations ceased
and the lesion on the tongue healed.

Subsequently, the prednisone was gradually decreased to a level of 40 mg. a day.
There were no additional hemorrhagic manifestations and the platelet count remained
at a low normal level. There was, however, an increase in the hypercalcuria, and osseous
metastases became visible on x-ray examination.

On the 79th hospital day the patient was discharged and followed in the Out-patient
Department on prednisone, 25 to 40 mg. per day. No further hemorrhagic episodes oc-
curred. Her hemoglobin remained at about 10 Gm.-% and her platelet count remained
within normal limits. She died at home approximately 3½ months after discharge, ap-
parently due to progressive metastatic cancer. No autopsy was performed.

Special Studies

Because of the clearly established precipitous fall in the patient’s platelet
count coinciding with the development of the bleeding manifestations, it
was apparent that practically all of the patient’s platelets had been destroyed
in a very short time. To demonstrate a mechanism by which this may have
occurred, the effects of the patient’s serum on normal platelets were studied.

In Vitro Studies

1. Methods: All glassware was siliconed. Blood for platelet and white cell
agglutination tests was drawn by Harrington’s method. Ten ml. was added
to 0.3 ml. 0.135 M (5 per cent) disodium ethylenediaminetetraacetate (EDTA)
in saline. In a few experiments, blood was added to one-ninth volume of
0.11 M sodium citrate or 0.1 M sodium oxalate or was decalcified by mixing
for five minutes with Dowex-50 beads in a proportion of three parts to one.
Platelet-rich plasma was obtained by slow centrifugation. Washed platelets
were prepared from blood added to one-ninth volume of 1 per cent EDTA.
The platelets were separated by differential centrifugation and washed three
times at 4 C. in isotonic saline. Serum was separated from blood which had
been placed at 37 C. overnight in glass tubes. Unless otherwise stated, all
sera were inactivated at 56 C. for 20 minutes.
Thrombin was prepared from human blood by the method of Biggs and Macfarlane and human fibrinogen by the method of Ware and Seegers. Tests for platelet agglutinins were carried out by Harrington's method by which the patient's serum is left overnight in the refrigerator with platelet-rich plasma in the presence of excess EDTA. Platelet-rich plasma was obtained from normal individuals either of type O or of the same blood type as the patients. Control serums were always tested concurrently. In the occasional instances in which the controls showed an appreciable amount of agglutination, the experiment was disregarded.

White cell agglutination was measured by the method of Brittingham and Chaplin by which the leukocytes are resuspended in 20 per cent bovine albumin.

Clot retraction was usually measured on a mixture of 0.2 ml. EDTA (5 per cent) platelet-rich plasma, 0.05 ml. inactivated serum and 0.025 ml. 0.1 M CaCl₂. When samples were clotted with thrombin instead of CaCl₂, oxalated, citrated or Dowex platelet-rich plasma was used, since EDTA platelet-rich plasma does not retract unless it is recalcified. After the contents had clotted firmly, the tubes were tapped to free the clot from the walls of the tube. The degree of retraction, which was readily reproducible, was estimated visually. In a system free of red cells, 4+ represents expression of serum equal to about 90 per cent of the total volume, 1+ represents expression of about 15 per cent, and zero represents absence of any visible retraction.

2. Results: (a) Platelet agglutination. As indicated in figure 1, the patient's serum obtained on the 24th, 31st and 35th days produced marked agglutination of platelets from the four normal individuals tested. Serum obtained on the 37th, 41st, 49th and 56th days gave less consistent results, producing definite agglutination against three normal donors in some tests but failing to produce any agglutination even against one of the same donors in other tests. Serum obtained on the 62nd day failed to produce agglutination in all five tests with the platelets of the same three donors. Serial titrations of the serum obtained on the 35th day, with serum from the normal donor of the platelet-rich plasma as a diluent, showed end points between 1:16 and 1:64. There was no evidence of a prozone. The agglutinin was not destroyed by inactivation at 56 C. for 20 minutes. It was not absorbed by BaSO₄, and was stable for at least one week at 4 C. and for 15 months at -20 C.

When platelet-rich plasma obtained from the patient during the remission of the thrombocytopenia was tested against her serum obtained at the time of maximal thrombocytopenia (24th and 31st days), no autoagglutinins could be demonstrated. This absence of autoagglutination did not seem attributable to direct inhibition of agglutination by corticosteroids, since addition of between 10 and 0.001 μg./ml. of compound F to the serum obtained on the 35th day failed to inhibit agglutination of normal platelets. Furthermore, platelets of two patients receiving 20 and 60 mg. of prednisone were agglutinated by the patient's serum. Because of the possibility that the failure to demonstrate autoagglutination might be due to coating of the platelets produced during remission by a "blocking" antibody, normal platelets from two different donors were first incubated for an hour either at 37 C. or 4 C.
with the patient’s serum drawn on the 62nd day. This produced no agglutination. A sample of the patient’s serum drawn on the 35th day was then added and the mixtures kept overnight at 4 C. as usual. Agglutination was as marked as with control platelets which had been exposed to normal serum instead of the potentially “blocking” serum of the patient. In other words, there was no evidence of “blocking” antibody in the patient’s serum drawn on the 62nd day; and, hence, there was no evidence that the failure to demonstrate auto-agglutination was due to in vivo coating of the patient’s platelets by blocking antibody.

(b) Platelet lysis. Five-hundredths ml. of the patient’s serum, obtained on the 35th day and inactivated, was incubated at 37 C. with 0.2 ml. normal, platelet-rich EDTA plasma and 0.025 ml. of 0.1M MgCl₂. Clearing of the suspension was observed within an hour, and observation with a phase microscope revealed “balloons” or platelet ghosts and free granules (fig. 2). No fibrin or platelet clumps and very few intact platelets were seen. In control samples prepared with normal serum, no ballooned platelets were ever seen; the platelets were either single (fig. 2) or clumped with a variable degree of fusion. A sample prepared with the patient’s serum but without the addition of MgCl₂ contained single intact platelets. When oxalated or citrated
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Fig. 3.—Degree of clot retraction in mixtures of 0.025 ml. J. P. serum, 0.2 ml. EDTA platelet-rich plasma and 0.025 ml. 0.1 M CaCl₂. From left to right, the serum used was obtained on Day 35, 37, 40, 55.

Platelet-rich plasma was used instead of EDTA plasma, platelet lysis in the presence of the patient's serum was less marked but occurred whether or not MgCl₂ was added.

Similar results were obtained with serum drawn on the 37th day. Serial titration of these sera, with normal serum as the diluent, revealed evidence of lytic activity up to and including the 1:8 dilution. Undiluted serum obtained on the 41st and 49th days produced lysis of only a few platelets. Sera from the 56th and 62nd days were entirely negative.

A heat labile plasma factor was required for this lysis. This was indicated by the observation that washed platelets placed in inactivated EDTA plasma, in the presence of the patient's inactivated serum and MgCl₂, did not undergo lysis; a control tube in which the plasma was not inactivated did show lysis.

No tests for lysis of the patient's own platelets were carried out.

(c) Inhibition of clot retraction. Serum obtained from the patient on the 24th, 31st and 35th days completely inhibited clot retraction when the serum and CaCl₂ were added to platelet-rich EDTA plasma obtained from any one of five normal donors or either of two patients receiving prednisone (fig. 3). It did not inhibit retraction of the patient's own platelet-rich plasma obtained during remission. When the serum obtained on the 35th day was titered, inhibitory activity could be demonstrated up to and including the 1:8 dilution. Retraction was moderately impaired when serum of the 37th day was used. Serum obtained on the 41st day and thereafter showed no such activity. The clot retraction inhibitor seemed unaffected by storage at −20 C. for 15 months, but lost considerable potency after one week at 4 C.

When the patient's serum was added to fresh whole blood from a normal individual, the degree of retraction depended upon the proportion of serum to blood. The addition of 0.025 ml. of serum to 0.5 ml. of blood did not cause
detectable inhibition; when 0.05 ml. of serum was used, retraction was somewhat inhibited, and when 0.1 or 0.2 ml. was used, retraction was negligible.

Special precautions were necessary to demonstrate inhibition of clot retraction when clotting was produced by the addition of thrombin rather than CaCl₂. However, if the patient's serum was pre-incubated with platelet-rich citrated plasma for one hour or if the platelet count in the mixture was reduced to about 100,000/cu.mm. by dilution with plasma marked inhibition was noted. Presumably, when thrombin is added at once rather than evolved slowly after recalcification, the retraction-promoting effect of thrombin is exerted before the patient’s serum reacts with the platelets. Similarly, the addition of the patient's serum to a mixture of plasma and washed platelets inhibited retraction of a thrombin-induced clot provided that the platelet count in the system was not too high. When buffered saline or 6 per cent neutralized human albumin* and fibrinogen were substituted for plasma, no inhibition of retraction was observed. This indicates that some plasma factor, possibly complement, is required for inhibition of clot retraction.

Histologic sections, stained with phosphotungstic acid–hematoxylin, were made of a normal retracted clot and of an unretracted clot formed in the presence of the patient's serum (fig. 4). The normal clot showed amorphous masses which we believe to be agglutinated, fused platelets; in contrast, the unretracted clot showed isolated, discrete bodies scattered throughout the fibrin meshwork.

*American Red Cross.
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### Table 1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Platelet Count</th>
<th>No. Transfusions</th>
<th>Platelet Agglutinin Titer</th>
<th>Platelet Lysin</th>
<th>Clot retr. Inhibitor</th>
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</thead>
<tbody>
<tr>
<td>J.P. 35th day</td>
<td>Ca Breast metastatic</td>
<td>0</td>
<td>4</td>
<td>1:16 to 1:64</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>J.P. 41st day</td>
<td>Ca Breast metastatic</td>
<td>1,250</td>
<td>4</td>
<td>Positive</td>
<td>trace</td>
<td>No</td>
</tr>
<tr>
<td>P.H.</td>
<td>Ca Cervix metastatic</td>
<td>375,000</td>
<td>9</td>
<td>1:128</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>D.R.</td>
<td>Chronic lymph. leuk.</td>
<td>5,000</td>
<td>40</td>
<td>1:64</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>E.N.</td>
<td>Ca Breast metastatic</td>
<td>21,000*</td>
<td>23†</td>
<td>Negative</td>
<td>Yes§</td>
<td>Yes**</td>
</tr>
</tbody>
</table>

*Bone metastases and treatment with 6-MP, DON and x-ray.
†Including 12 administered one or two days prior to sample.
§Against one normal but not against a second normal.
**Against J. P. and one out of five normals only. The normal individual whose retraction was inhibited was the same individual whose platelets were lysed by the patient’s serum.

(d) Tests on siblings. Although the clinical course of the patient suggested that autoagglutination was responsible for her thrombocytopenia, the patient's serum had no in vitro effect upon the platelets which she produced during remission. This suggested the possibility that an isoagglutinin might be involved. Since the patient's serum reacted with the platelets of all individuals tested (5 normal and 2 prednisone-treated), and since only a limited amount of her serum was available, it seemed advisable to test her serum against the platelets of her three surviving siblings. Platelet agglutination and lysis and inhibition of clot retraction were observed in all three instances. While this observation is not conclusive, it does support the thesis that the patient's platelets were not of some uncommon antigenic type.

(e) Leukoagglutinins. Serum taken on the 35th day caused agglutination of the white cells of three normal individuals; the titer was 1:2. Earlier samples were not tested. All subsequent samples were also positive, although agglutination was less marked in the last two samples, taken on the 56th and 62nd days. Control tests with sera obtained from normal individuals were uniformly negative.

(f) Comparison with other patients. The sera of 12 other patients who, like J. P., had repeated pyrogenic nonhemolytic transfusion reactions were studied. The sera of three of these patients affected the platelets (table 1). Two of the patients (P. H. and D. R.) had platelet agglutinins with titers higher than J. P.'s. Neither of these patients, however, had a demonstrable platelet lysin or clot retraction inhibitor. The serum from both patients agglutinated normal white blood cells. Serum from E. N. inhibited clot retraction of platelet-rich plasma from J. P. and from one normal individual, but had only a slight inhibitory effect on clot retraction from a second normal individual, and no effect when tested against three other normals. Serum from this patient failed on several occasions to produce platelet agglutination, even when tested against the platelets active in the clot retraction inhibition...
test. However, it did produce lysis of the platelets of the normal individual whose clot retraction was inhibited and failed to produce lysis of the platelets of a normal individual whose retraction was not inhibited. Unfortunately, no more serum was available for further tests.

Tests for platelet agglutinins were carried out in 40 other patients with unexplained thrombocytopenia and plentiful megakaryocytes. The sera of six of these patients were positive for platelet agglutinins (table 2), but none had a platelet lysin or clot retraction inhibitor.

**In Vivo Studies** (Fig. 5)

One hundred ml. of blood were drawn from patient J. P. into ACD solution on the 31st hospital day and were transfused the same day into a hematologically normal recipient. The platelet count of the recipient fell from 350,000 to 25,000 within 15 minutes of the start of the transfusion, and she developed a chill and slight elevation of temperature. The platelet count did not return to a normal level for 12 days; 150 ml. of the patient's blood drawn 18 days later were transfused into the same recipient. On this occasion the fall in the recipient's platelet count was less marked and less prolonged, and there was a milder transfusion reaction.

**Discussion**

Serum from patient J. P. was unusual since it not only produced agglutination of platelets from normal individuals but caused platelet lysis and inhibition of clot retraction as well. Platelet lysins accompanying platelet agglutinins have been mentioned occasionally but, except in drug purpuras, the methods used for their detection have usually been either nonspecific or unreliable. The production of thrombocytopenia in an individual receiving a transfusion of blood from a thrombocytopenic patient can as readily be ascribed to platelet agglutination and sequestration as to direct platelet lysis. As Dausset and Malinvau have pointed out, a decrease in the platelet count or in the turbidity of a platelet suspension in vitro may result from platelet agglutination alone.
Complement is apparently necessary for platelet lysis in the antibody systems thus far studied.\textsuperscript{2,10,11} Hence, tests carried out with plasma containing anticoagulants are unreliable, since oxalate, citrate, EDTA and heparin are all anticomplementary.\textsuperscript{13-15} It is impractical to add untreated serum to the platelet suspensions as a source of complement because the serum clotting factors will affect the platelets. Consequently, some method must be found to overcome the anticomplementary effect of the anticoagulants used. Since the anticomplementary properties of EDTA are attributable to its chelation of magnesium and calcium ions,\textsuperscript{14} in the platelet lysis test described in this paper MgCl\textsubscript{2} was added to a mixture of the patient’s serum and EDTA platelet-rich plasma. Calcium was not added because of its effect on blood clotting and did not seem to be essential for platelet lysis. After the addition of magnesium, no macroscopic or microscopic traces of fibrin were found. Platelet destruction was indicated by clearing of the mixture after one hour at 37 C. or, even better, by the marked morphologic changes exhibited by the platelets. No such changes occurred when the EDTA plasma was previously inactivated by heating to 56 C. for 20 minutes. Thus, this test should prove to be a useful and simple method for demonstrating platelet lysis.

The use of this test on several patients with platelet agglutinins demonstrated that the presence of platelet lysin and agglutinin were unrelated. For example, a platelet lysin was present in the serum of patient J. P. and was absent in the sera of two other patients with platelet agglutinins of higher titer; con-
versely, a platelet lysin was demonstrated in the serum of patient E. N., but no platelet agglutinin could be shown.

Dausset\textsuperscript{16} and Stefanini\textsuperscript{17} have each described a clot retraction inhibitor in the serum of a patient with thrombocytopenia and platelet agglutinins. Since the agglutinins were very potent in these patients, it was suggested that the inhibition of retraction was a consequence of platelet agglutination. In a clot prevented from retracting by the addition of J. P.'s serum, however, histologic sections failed to demonstrate platelet agglutination. Furthermore, inhibition of retraction was not correlated with the presence or titer of platelet agglutinin, since the serum of two patients with a higher titer of agglutinins than patient J. P. failed to inhibit clot retraction, whereas the serum of patient E. N. inhibited retraction of the platelet-rich plasma of two donors, yet failed to agglutinate the platelets of these or other donors. On the other hand, the presence of platelet lysin correlated well with inhibition of clot retraction. Both were present in J. P.'s serum. Serum from E. N. produced both lysis and clot retraction inhibition using the platelets of one normal donor; it failed to produce either when tested against platelets from another donor. Unfortunately, there was insufficient serum to test for lysis against other donors, and it was not possible to test for lysis of J. P.'s platelets by E. N.'s serum.

If platelet lysin is responsible for inhibition of clot retraction, one would expect the platelets in the histologic section of the unretracted clot to be lysed. It is impossible to state whether the single bodies seen in the section represent platelet ghosts remaining after lysis had occurred, or whether they are intact platelets. In any event, they are present in approximately the number to be expected, since there are about 2,500 “platelets”/sq. mm. in the 8 micron sections, which agrees well with the initial platelet count of about 300,000/cu.mm.

Dausset was unable to demonstrate inhibition of clot retraction when samples of citrated platelet-rich plasma were clotted with thrombin rather than with CaCl\textsubscript{2}.\textsuperscript{18} We found that the conditions were more critical but that inhibition did occur in thrombin clots, provided the platelet count was not too high or the patient's serum was incubated with the platelet-rich plasma before the addition of thrombin. These findings can be explained by the fact that although citrate is anticomplementary, it does not completely abolish complement activity.\textsuperscript{13} The importance of complement in the inhibition of clot retraction was further indicated by the observation that inhibition could not be demonstrated in a plasma-free system containing the patient's inactivated serum, buffer or albumin, fibrinogen, washed platelets and thrombin. This apparent dependence of clot retraction inhibition on the presence of complement further suggests the identity of the clot inhibitor factor and the platelet lysin; this is in contradistinction to the platelet agglutinin which does not require complement.

There is no question that the thrombocytopenia which developed in patient J. P. was due to an abnormally rapid destruction of her own platelets. In view of recent estimates of normal platelet life span,\textsuperscript{18,19} the fall in the platelet count from 184,000 per cu. mm. to zero in less than three days cannot be accounted for in any other way. The presence of potent antiplatelet fac-
tors in the patient's serum at this time would seem to demonstrate the mechanism by which the thrombocytopenia developed. Furthermore, the gradual disappearance of these factors from her serum correlated with the recovery of her own peripheral platelet count. It was surprising, therefore, that serum obtained from the patient during the period of her thrombocytopenia failed to produce agglutination or to inhibit clot retraction when tested against the patient's own platelets, produced while she was under corticosteroid therapy. This has not been the experience of other investigators. When the platelet count of the patient was sufficiently high to conduct tests, Dausset,2 Stefanini26 and Harrington4 found autoagglutination in every instance of thrombocytopenia in which the patient's serum agglutinated platelets from normal individuals. In fact, in some cases, autoagglutination was demonstrable even when the patient's serum failed to agglutinate the platelets of other persons. Although it cannot be denied that the circulating platelet agglutinin detected in the serum of our patient, J. P., may have been an isoantibody and quite unrelated to the patient's thrombocytopenia, this seems improbable in view of the established relationship between the disappearance of the agglutinin and the thrombocytopenia. It is quite conceivable that the failure to demonstrate autoagglutination and inhibition of clot retraction was due to some modification of the patient's platelets, formed as she recovered, so that they were no longer susceptible as normal platelets were. Steroid administration itself was not responsible for the absence of autoagglutination, since the patient's serum was able to produce agglutination and inhibition of clot retraction when tested against the platelets of two patients who were receiving 20 and 60 mg. of prednisone daily. Attempts to demonstrate a "blocking" mechanism in vitro were unsuccessful, but this does not eliminate the possibility that the patient's platelets were coated with "blocking antibody" by some in vivo process which could not be reproduced in vitro. Unfortunately, we do not know whether sera from other patients with platelet agglutinins would have agglutinated the patient's platelets, since none were available at the time. However, serum from patient E. N. completely inhibited retraction of J. P.'s platelet-rich plasma, showing that J. P.'s platelets could react to the clot retraction inhibitory substance which we believe to be a platelet lysin.

The observations made in our patient resemble the findings in many patients with leukopenia whose serum agglutinates the white cells of others but not their own. Dausset has reviewed the reasons for believing that the leukopenia is the result of autoimmune mechanisms despite the failure to demonstrate autoagglutination,2,5 although the situation is complicated by the development of isoagglutinins for leukocytes as a result of multiple transfusions.8,21,22

Although no biopsy or autopsy was carried out, it is very probable that patient J. P. had metastatic carcinoma of the breast. The occurrence of platelet agglutinins in five patients with carcinoma and thrombocytopenia has been demonstrated by others, but details were not presented.1,4,5 Three of these patients had demonstrable autoagglutinins2; the others were not tested because of their low platelet counts.

White cell agglutinins have been implicated as a cause of nonhemolytic
transfusion reactions. The frequent occurrence of reactions in normal individuals receiving blood from patients with idiopathic thrombocytopenic purpura suggests that platelet agglutinins may also cause reactions. Transfusion of blood from J. P. into a hematologically normal recipient produced not only thrombocytopenia but also chills and fever. Unfortunately, the white count of the recipient was not followed since it was not recognized at that time that J. P. had leukoagglutinins as well as platelet agglutinins. If platelet and/or leukoagglutinins are considered the cause of J. P.’s transfusion reactions, she must have had these agglutinins prior to her first known transfusion. In our experience and that of others, however, platelet or white cell agglutinins are not found in all patients who have pyrogenic transfusion reactions, and her transfusion reactions may have been the result of serum haptoglobin or unknown factors. It is of interest, however, that the patient ceased to have pyrogenic reactions as her thrombocytopenia began to diminish.

SUMMARY

A case is reported of thrombocytopenia developing suddenly in a patient with metastatic carcinoma. Studies of this patient’s serum revealed a platelet agglutinin presumed to be related to the development of the thrombocytopenia in spite of the failure to demonstrate autoagglutination. In addition, a technique is described for the detection of a platelet lysin and clot retraction inhibitor, both of which could be demonstrated in this patient’s serum. The identity of the lysin and retraction inhibitor is suggested.

Studies on the sera of other patients showed a dissociation between the occurrence of platelet agglutinin on the one hand and of platelet lysin and retraction inhibitor on the other.

SUMMARIO IN INTERLINGUA

Es reportate un caso de thrombocytopenia que se disveloppava subitemente in un patiente con carcinoma metastatic. Studios del sero del patiente revelava le presentia de un agglutinin plachettal. Isto eseva considerate como relationate al disveloppamento del thrombocytopenia in despecto del impossibilitate de demonstrar autoagglutination. In plus, un technica es descripte pro le detection de un lysina plachettal e un inhibitor del retraction de coagulo, le quales eseva ambes demonstrabile in le sero del patiente in question. Es presentate suggestiones relative al identitate del lysina e del inhibitor.

Studios in le seros de altere patientes monstrava un dissociation inter le occurrientia de agglutinin plachettal de un latere e de lysina plachettal e inhibitor del retraction del altere latere.

REFERENCES


DEVELOPMENT OF THROMBOCYTOPENIA


Thrombocytopenia with a Circulating Platelet Agglutinin, Platelet Lysin and Clot Retraction Inhibitor

MARJORIE B. ZUCKER, ALLYN B. LEY, JENNIE BORRELLI, KLAUS MAYER and JORGE FIRMAT