Specificity of Lytic Factors for Erythrocytes, Leukocytes and Platelets in a Case of Pancytopenia

By Y. Matot, E. Elian, D. Nelken and A. C. Nevo

Mechanisms analogous to those operating in acquired hemolytic anemia have been demonstrated in cases of "idiopathic" thrombocytopenic purpura1-3 and more recently in certain cases of leukopenia.4, 5 Increased destruction of the three respective blood cell types by autoimmune antibodies may therefore be a mechanism common to all of these conditions. Consequently, combined cytopenias, in which more than one blood cell type is affected in the same individual, are of interest.

An antileukocyte factor has recently been demonstrated in some cases of pancytopenia. In the case of Kissmeyer-Nielsen4 and in that of Moeschlin5 the evidence was derived from transfusion experiments. Dausset8 using the in vitro agglutination method found leukoagglutinating activity in 48 sera. Of these, 8 sera contained antibodies for another cell type, either erythrocytes or platelets, whereas antibodies for all three cell types were found in 3 sera.

In a case of pancytopenia, reported briefly in the present communication, increased destruction of all three blood cell elements was demonstrated in vivo. In addition, the antileukocyte and antiplatelet factors involved were shown by in vitro methods to be agglutinins. This case afforded an opportunity to study the problem: is increased destruction of more than one blood cell element in the same individual due to a single (or to multiple) cell-type specific factors?

Case History

A 6 year old girl was admitted because of easy bruising and nose bleeds which she had had since the age of one year. She also suffered from frequent infections which usually coincided with exacerbations of the bleeding tendency.

Physical examination was negative except for ecchymoses all over the skin and a few petechiae in the oral mucosa. The spleen and liver were not palpable. The red cell count was 2.3 million per cu.mm., the hemoglobin 6.7 Gm. per cent, reticuloocytes 2.8 per cent. The platelets numbered 33,000 per cu.mm. and the white cells 3,300 with 12 per cent granulocytes. Clotting time was 5 minutes (Lee-White), clot retraction was absent after 24 hours. The bleeding time was over 30 minutes (Duke) and the capillary fragility was markedly increased (Rumpel-Leede). Prothrombin time was normal (Quick) and fibrinogen was 210 mg. per 100 ml. The serum bilirubin level was not elevated and urinary urobilinogen was not increased.

Bone marrow, on repeated puncture, was moderately hypocellular with a myeloid-erythroid ratio of 3:1. The red series showed a relative increase in young immature forms, whereas the distribution of white precursor cells appeared to be within normal limits. A few young forms of the megakaryocytic series, but no mature megakaryocytes, were found. Plasma cells and histiocytes were found in increased numbers.

Cortisone, 100 mg. daily, was given orally for 14 days. During treatment with cortisone the red cell count rose from the pre-treatment level of 2.0 million to 2.8 million and the...
hemoglobin from 5.9 to 8.1 Gm. The white cell count reached a maximum of 8,500, 20 per cent of which were granulocytes. The platelet count rose from 22,000 to 67,000 at the end of the first week of cortisone treatment and thereafter gradually fell to 37,000 at the end of the second week. The bleeding tendency decreased in severity during the treatment with cortisone.

During the nine months following discharge, the child was followed in the Outpatient Clinic and re-admitted twice for recurrent respiratory infections with aggravation of the bleeding tendency. During this period the red cell count fluctuated between 2.2 and 2.7 million, and the hemoglobin between 6.0 and 8.4 Gm per cent. The platelet count varied between 25,000 and 85,000, but was usually about 40,000. The total white cell count varied between 2,500 and 7,000, and the number of granulocytes was about 500 per cu.mm., occasionally ranging as low as 280 or as high as 2,000.

**Experimental**

In the investigation of this case, both in vivo and in vitro methods were used to demonstrate increased destruction of each of the three blood cell types. Cross absorption studies eliminated the possibility of a single factor being involved in the destruction of all three cell types. In an attempt to obtain information on the mode of action of antplatelet and antileukocyte antibodies, microelectrophoretic and phagocytic studies were conducted.

**Demonstration of Increased Destruction of Blood Cells**

**Erythrocytes**

The osmotic and mechanical fragility of the patient's erythrocytes was normal. The erythrocytes were group O Rh±Rhr-. The Coombs test, direct and indirect, was negative. No autoagglutinins or autohemolysins were found at 6, 20 or 37 C.

The survival of normal ON cells transfused into the patient was studied by the differential agglutination method as adapted for finger blood by Kaplan and Zuelzer. The survival of the transfused cells was markedly shortened (fig. 1).

**Leukocytes and Platelets**

1. **Transfusion experiments.** One hundred ml. of the patient's plasma were transfused into a normal infant of blood group O, whose weight was 6 Kg. Serial
FIG. 2.—Leukocyte counts and absolute granulocyte counts in a normal infant transfused with 100 ml. of the patient's plasma.

Leukocyte and platelet counts were done on the recipient following the transfusion. On the evening before the day of transfusion the recipient developed a respiratory infection with fever and leukocytosis. His white cell count, repeated three times during the few hours preceding the transfusion, ranged between 20,000 and 21,000. Following transfusion there was a steady drop in the white cell count, which reached 10,000 in three hours (fig. 2). The fall in the granulocytes was even more marked. They decreased from 12,000 before transfusion to 3,700 immediately following it and remained at a low level for the entire three hour period of the experiment.

Platelet counts were done with Dameshek's indirect method.19 Repeated platelet counts on the recipient on the two days preceding transfusion ranged between 350,000 and 400,000. There was a gradual drop in the platelet count from a pretransfusion level of 350,000 to 127,000 three hours following transfusion. The platelet count remained low for the three following days, after which it gradually rose to 320,000 on the seventh day following transfusion (fig. 3).

A transfusion of 100 ml. normal O bank plasma given to the same recipient four weeks later produced no significant changes in the leukocyte and platelet counts. The white cell count before transfusion was 13,600 and varied between 12,200 and 13,000 during the three hours following the transfusion. Similarly, the platelets before transfusion were 385,000 and after it 300,000.
The indirect Coombs test for platelets was performed according to a technic evolved by Gurevitch and Nelken, which agrees closely with the recently published technic of Ruggieri. Using this delicate method in the study of a series of cases of thrombocytopenia, Gurevitch and Nelken (unpublished data) found it reproducible whenever positive.

2. In Vitro Agglutination

a. White Cells. The technic described by Dausset for the agglutination of leukocytes was followed with the following modifications: Dextran was used for the differential sedimentation of red cells and the leukocytes in the supernatant plasma were concentrated by light centrifugation to 15,000–20,000 per cu.mm. The leukocytes and control sera used were all group O, the patient’s blood group.

The leukocyte agglutination experiments were performed on four separate occasions with sera obtained from the patient over a period of six months and kept in the frozen state. These sera included one obtained during cortisone treatment. All sera gave 1+ to 4+ positive agglutination reactions (fig. 4). No correlation was found between the intensity of the reaction and the degree of leukopenia or granulocytopenia existing at the time the sera were obtained. No leukoagglutination was observed with several sera from normal persons and from patients with acute leukemia, aplastic anemia and idiopathic thrombocytopenic purpura.

b. Platelets. Saline suspensions of normal washed group O platelets, prepared as described by Gurevitch and Nelken, were incubated for one hour at 37 C. with sera obtained from the patient. No agglutination or lysis of the platelets was found under these conditions. However, when the technic of the indirect Coombs test for platelets* was used, a strongly positive agglutination reaction was repeatedly observed.

* The indirect Coombs test for platelets was performed according to a technic evolved by Gurevitch and Nelken, which agrees closely with the recently published technic of Ruggieri. Using this delicate method in the study of a series of cases of thrombocytopenia, Gurevitch and Nelken (unpublished data) found it reproducible whenever positive.
Fig. 4. A. Agglutination of normal leukocytes by patient's serum. B. Control (normal serum).
LYTIC FACTORS IN PANCYTOPENIA

Table 1.—Agglutination reactions of normal leukocytes and platelets with patient's serum after absorption with erythrocytes, leukocytes or platelets

<table>
<thead>
<tr>
<th>Serum</th>
<th>Leukocyte agglutination</th>
<th>Platelet agglutination (indirect Coombs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient's serum:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unabsorbed</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Absorbed with erythrocytes</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Absorbed with papaine-treated erythrocytes</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Absorbed with leukocytes</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Absorbed with platelets</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>Normal serum</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

CROSS ABSORPTION STUDIES

Agglutination tests on normal leukocytes and indirect Coombs tests on normal platelets were done with the patient's serum after absorption with erythrocytes, leukocytes or platelets.

Absorption of sera was carried out in the following manner: to 0.3 ml. aliquots of the patient's serum, inactivated at 56°C for 30 minutes, were added:

1. 0.3 ml. of packed group ORh- platelets
2. 0.3 ml. of packed ORh- erythrocytes
3. 0.3 ml. of papaine treated ORh- erythrocytes
4. 0.3 ml. of packed ORh- leukocytes

The serum-cell mixtures were allowed to stand at 20°C and shaken at intervals. At the end of 15–20 hours the mixtures were centrifuged and the serum pipetted off.

Results obtained with the absorbed sera are shown in table 1. It will be seen that leukocytes were agglutinated by all but the leukocyte-absorbed sera. Similarly, the indirect Coombs test for platelets was positive with all but the platelet-absorbed sera.

ELECTROPHORETIC STUDIES

The electrophoretic mobility of normal leukocytes and platelets after exposure to the patient's serum was studied in a Northrop-Kunitz type microelectrophoresis chamber.

Washed suspensions of normal group O leukocytes and platelets, prepared in the same manner as for the agglutination experiments, were incubated at 37°C for 40 minutes with equal volumes of the patient's serum, previously inactivated at 56°C. The cells were then washed once and resuspended in saline-veronal buffer, pH 7.3.*

The electrophoretic mobility of platelets incubated in the patient's serum did not differ from that of platelets incubated in normal serum (table 2). The difference between the mobilities of leukocytes incubated in the patient's serum and in normal serum was not significant. It was slightly larger than the experimental

* Buffered sodium chloride solution of pH 7.3 and ionic strength 0.15 was prepared by adding 40 ml. of 0.1 N veronal-HCl buffer to 1 liter of 0.9 per cent saline.
Table 2.—Electrophoretic mobilities of normal leukocytes and normal platelets exposed to the patient's serum

<table>
<thead>
<tr>
<th>Blood Cell Types</th>
<th>Mobility μ/sec./volt/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets:</td>
<td></td>
</tr>
<tr>
<td>suspended in saline</td>
<td>-0.90</td>
</tr>
<tr>
<td>incubated with normal serum</td>
<td>-0.90</td>
</tr>
<tr>
<td>incubated with patient's serum</td>
<td>-0.91</td>
</tr>
<tr>
<td>incubated with normal serum, washed and resuspended in saline</td>
<td>-0.88</td>
</tr>
<tr>
<td>incubated with patient's serum, washed and resuspended in saline</td>
<td>-0.875</td>
</tr>
<tr>
<td>Erythrocytes (reference)</td>
<td>-1.10</td>
</tr>
<tr>
<td>Leukocytes:</td>
<td></td>
</tr>
<tr>
<td>incubated with normal serum</td>
<td>-0.585</td>
</tr>
<tr>
<td>incubated with patient's serum</td>
<td>-0.605</td>
</tr>
<tr>
<td>Erythrocytes (reference)</td>
<td>-1.00</td>
</tr>
</tbody>
</table>

error under optimal conditions of measurement. However, because of their large size, leukocytes sediment rapidly, so that relatively few valid readings were made at the stationary level. Under such conditions the difference was within the limits of experimental error.

Phagocytosis Experiments

A possible inhibition of phagocytosis due to the toxic action of the patient's serum on leukocytes was sought. Phagocytosis experiments were conducted according to a method previously described. Normal group O leukocyte suspensions were incubated with the patient's serum for varying lengths of time up to 1 hour and 30 minutes, and a suspension of starch particles was added to the serum-cell mixture at the end of the incubation period. A drop of this mixture was then placed on a slide, covered and sealed with vaseline, and incubated at 37 C. for 10 minutes.

Phagocytic activity observed in mixtures containing the patient's serum paralleled that in mixtures containing normal serum. In both, phagocytic activity was 100 per cent when phagocytosis was allowed to proceed without previous incubation of the leukocytes in either the patient's or normal serum, and tended to decrease with the length of time of pre-incubation. A toxic action of the patient's serum on leukocytes, expressing itself in inhibition of their phagocytic function, was therefore not demonstrated under the conditions of our experiment.

Discussion

The main clinical feature of this case was purpura due to thrombocytopenia. Anemia and leukopenia were discovered in the course of investigation in the hospital. However, the leukopenia probably accounted for the history of increased susceptibility to infection. In its clinical and hematological features, as well as in its lack of response to cortisone, our case resembled the cases of chronic idiopathic pancytopenia described in adults by Dausset.

The pancytopenia in our case was shown to be due, at least in part, to in-
creased destruction of the respective blood cell types by factors in the patient's plasma. A lytic factor for leukocytes and platelets respectively was found in transfusion experiments. Agglutination studies in vitro corroborated this finding and demonstrated the antibody nature of the factor (or factors) concerned. The survival of normal erythrocytes transfused into the patient was markedly shortened. However, no autohemagglutinins or lysins could be demonstrated with current methods, including the Coombs technique.

Although in the majority of cases cytopenias due to increased destruction present as monocytopenias, evidence of active or latent lytic mechanisms for the apparently nonaffected systems can be obtained with sufficient frequency to render the possibility of chance coincidence most unlikely. The frequent association of leukopenia and hemolytic anemia, or at least of red cell sensitization, with idiopathic thrombocytopenic purpura was pointed out by Evans, who postulated increased destruction as a common denominator for all three cytopenias. Such increased destruction of more than one cellular element in the same individual may be accounted for by a single antibody reacting with antigenic elements common to all affected blood cell systems or by multiple cell-type specific antibodies.

Our cross absorption studies provide experimental evidence for multiple antibodies being involved in the production of combined cytopenias. They have established that at least two distinct factors, each specific for either leukocytes or platelets are at play. As the antileukocyte and antiplatelet activity of the patient’s serum was not affected by absorption with erythrocytes, the anticyrthrocyte factor would also seem to be specific and distinct from the antileukocyte and antiplatelet factors.

No other experimental work bearing on the problem of a single versus multiple antibodies in combined cytopenias is known to us. On theoretical grounds a single antibody, by being the simpler solution, may at first glance seem more acceptable. The demonstration of ABO blood group antigens in platelets and probably also in leukocytes seems to support the assumption of a single antibody common to all three cell systems. However, ABO isoagglutinins are not involved in the production of immunocytopenias, and the fact that erythrocytes, leukocytes and platelets share the ABO antigens does not necessarily mean complete antigenic identity of the three blood cell systems. Furthermore, if a single antibody having affinity for all three systems was involved in the production of combined cytopenias, one would expect to see them much more frequently than they actually occur, and, as Evans observed, there would have to be a closer correlation between the severity of a hemolytic process and the degree of any associated thrombocytopenia or leukopenia.

A single antibody acting on all three cell systems is not necessarily the only explanation for the observation that combined cytopenias occur more frequently than would be determined by pure chance. When conditions favorable to auto-sensitization, whatever their nature, exist, they may quite conceivably affect more than one tissue. In this case the frequent occurrence of combined cytopenias would be due to the production of more than one antibody in response to such conditions.

Coombs and Race have shown that in red cells sensitized in vitro with Rh
antibody there occurs a change in the electrophoretic mobility. In our experiments, no such alteration of electrophoretic mobility of leukocytes or platelets exposed to the patient’s serum was found. However, determinations of electrophoretic mobilities of leukocytes and platelets are of interest in themselves because of the paucity of information on this subject in the literature. For example, the mobility of granulocytes in our experiments, 0.585–0.605 μ/sec./volt/cm, is considerably higher than the value of 0.333–0.435 μ/sec./volt/cm, found by Robineaux and Bazin. With such large particles as leukocytes it is difficult to obtain great accuracy in the horizontal Northrop-Kunitz chamber. Still the variation is less than ±5% and the discrepancy is therefore not likely to be due to the measuring technic itself. Platelets, on the other hand, proved to be most suitable for electrophoretic measurements and gave close and reproducible readings.

Testing leukocytes for their phagocytic ability after exposure for varying lengths of time to the action of a leukotoxic serum may prove a useful tool for measuring the time factor in the reaction between cell and antibody. No differences in phagocytic behavior between cells treated with the pathologic serum and those treated with normal serum were found under the conditions of our experiment. By varying these conditions, such as the length of time and temperature of incubation or the substrate for phagocytosis, such differences might be detected and have indeed been reported.

**SUMMARY**

A case of chronic idiopathic pancytopenia in a young girl is presented, in which the pancytopenia was shown to be due to increased destruction of all 3 blood cell types. Antileukocyte and antiplatelet antibodies were demonstrated by transfusion methods as well as by in vitro agglutination, while differential agglutination provided evidence of a plasma factor causing increased red cell destruction.

Cross absorption experiments demonstrated the presence in the patient’s serum of at least 2 separate and distinct antibodies, specific for leukocytes and platelets respectively.

Observations on the phagocytic behavior of leukocytes and on the electrophoretic mobility of leukocytes and platelets exposed to the patient’s serum are reported.

**S Sommaio in Interlingua**

Es presentate un caso de chronic pancytopenia idiopathiche in un juvine puera. Il esseva demonstrate que le pancytopenia esseva causate per accrescite destruc- tion de omne tres typos de cellulas sanguinee. Le presentia de anticorpores anti-leucoctytic e antiplachettal esseva demonstrate per methodos de transfusion e etiam per agglutination in vitro, durante que agglutination differential indicava le existentia de un factor del plasma que causava accrescite destrucion del erhythroctos.

Experimentos a absorption cruciate demonstrava le presentia in le sero del paciente de al minus 2 anticorpores, separate e distincte, le un specific pro leuco- cytos e le altere pro plachettas.

Es etiam reportate observationes super le conducta phagocytic de leucocytes
LYTIC FACTORS IN PANCYTOPENIA

e super le mobilitate electrophoretic de leucocytes e plachettas, que habeva essite exponite al sero del paciente.

REFERENCES


Specificity of Lytic Factors for Erythrocytes, Leukocytes and Platelets in a Case of Pancytopenia

Y. MATOTH, E. ELIAN, D. NELKEN and A. C. NEVO