Leukoagglutinins

V. Leukoagglutinins in Chronic Idiopathic or Symptomatic Pancytopenia and in Paroxysmal Nocturnal Hemoglobinuria

By J. Dausset, A. Nenna and H. Brecy

In recent years immunohematology has been advanced by studies concerning the presence of antibodies in the sera of patients with acquired hemolytic anemias and thrombocytopenic purpuras. It has therefore seemed logical to undertake comparable studies of the leukocyte series, and to attempt to demonstrate the presence, in some syndromes with leukopenia, of substances active upon leukocytes. Leukolysins have been shown to be present in certain granulocytopenias by Franke, Oliva and Furbetta, and Ninni. Wiard and Robbins have described one case in which a powerful leukocidin was present. Recently, Moeschlin and Wagner observed that the serum of a patient with granulocytopenia resulting from pyridoxin hypersensitivity was capable, during the hours immediately following ingestion of the drug, of agglutinating normal human leukocytes in vitro. Injecting the whole blood of this patient into a normal individual produced a rapid fall in the white count; whereas, injecting normal blood failed to affect it. Dausset and Nenna have studied the serum of a patient with acute aleukemic leukemia; this serum was shown to be capable of provoking a strong agglutination of several samples of normal leukocytes. Subsequently, they have described two and six cases of patients with leukopenia in whose sera a leukoagglutinin appeared independent of any sensitization or intoxication; therefore, this represented a phenomenon different from that described by Moeschlin and Wagner. Tullis and Twibble observed that the sera of patients with granulocytopenia or aleukemic leukemia sometimes possess the property of inducing leukocyte agglutination. Miescher has studied a case of chronic granulocytopenia in which the serum injected into rabbits provoked a profound reduction in the circulating leukocytes. Recently, Goudsmit and van Loghem have reported a study of ten sera possessing the same property.

Since our first publication, we have systematically studied the leukoagglutination phenomenon following a technic which we have developed, and we have encountered nineteen sera which present this property. The sum of our observations, serologic and clinical, is presented here.
Materials and Techniques

Materials

1. Sera: More than two thousand sera were tested, including those of normal donors; five hundred sera of patients with various conditions, of which one hundred and two had leukopenia; of these one hundred and two, nineteen sera demonstrated leukoagglutination.

2. Leukocytes: The leukocytes were at first obtained from three hundred normal donors, each giving a single sample. Later seventeen donors were used at regular intervals for all tests. It was established that these sera were incapable of agglutinating the leukocytes of compatible blood groups.

Technics

1. The test for leukoagglutination. This test was carried out by adding the serum to be studied to a suspension of leukocytes as completely free of red cells as possible, and completely free of platelets. Defibrinated blood from normal donors was used as a source of leukocytes in order to avoid using anticoagulants and in order to eliminate the platelets. The leukocytes were obtained by sedimentation from the defibrinated blood after the addi-
LEUKOAGGLUTININS

Fig. 2.—Slightly positive (+) leucoagglutination test

tion of polyvinyl pyrrolidone,* taking care to use blood compatible with the serum to be tested, in order to avoid interference by agglutination of the red cells.

2. Preparation of the leucocyte suspension. Thirty cc. of blood drawn under aseptic conditions was agitated gently for about 10 minutes in an Erlenmeyer flask containing ten glass balls 4 or 5 mm. in diameter. The resulting fibrin clot was discarded; one-fifth of the original volume of polyvinyl pyrrolidone (Subtoxan Special) was added and the blood allowed to sediment for 1.2 hr. at 37 C. in a tube inclined at an angle of 45 degrees. The faintly pink supernatant serum was then carefully drawn off. It contained 5 to 10,000 leukocytes per cu. mm. and 50 to 100,000 red cells per cu. mm. No platelets were seen. It was sometimes necessary to increase the leucocyte count of the suspension by centrifugation (3 minutes at 1000 rpm). The upper half of the plasma was discarded, and the packed cells resuspended in saline. The leucocytes on smear were well separated and preserved their normal morphology. Their phagocytic function was likewise intact. It was found essential to use the leucocytes within a few hours of collection and to keep them at a constant temperature of 4 C.

3. Reaction proper. To 0.10 cc. of the serum to be tested, previously heated for 10 to 30

* Dextran, which has a high molecular weight (100,000), may be used. It produces more rapid sedimentation (Goudsmit and van Loghem (19)).
minutes at 56 C., 0.05 cc. of leukocyte suspension was added. After being shaken, the tubes were left in the incubator for 1 hour at 37 C.

4. Reading the reaction. After incubation, 0.10 cc. of 1 per cent acetic acid was added to each tube. The mingled contents of the tube were placed on a glass slide, forming a large drop about 1 cm. in diameter and 1 mm. in thickness. This was then examined under the microscope at low (30 X) magnification. In a negative reaction the leukocytes were seen to be well separated and regularly distributed in an even layer (fig. 1). In a positive reaction, the leukocytes formed rounded, compact conglutinations, far apart, with very few free cells in the spaces between (figs. 2, 3, 4). The intensity of the reaction was noted and graded −, ±, +, ++, ++++. Sometimes reading the reaction was difficult because of a paucity or superabundance of cells; however, suspensions of 5 to 10,000 leukocytes per cu. mm. allowed easy reading.

5. Alternative techniques discarded. Before settling on the above-described technic, the following processes were tried: (a) Addition of anticoagulants was abandoned since heparin is known to give false agglutination. Oxalate, citrate, and sequestrene were observed to produce partial inhibition of the leukoagglutination reaction. (b) Slow centrifugation and pipetting of the buffy coat failed to provide a large enough volume of white cell suspension.
LEUKOAGGLUTININS

Fig. 4.—Red cell life-span curves (Ashby's technic) in one case of chronic idiopathic pancytopenia (broken line), and one case of paroxysmal nocturnal hemoglobinuria (open circles).

and included too many red cells. (c) The originally described concentration of acetic acid (0.5 per cent) was increased to 1 per cent in order to eliminate any interference by red cells which escaped hemolysis. (d) Silicone tubes were found to give false negative results. (e) Hemolysis of the remaining red cells in the WBC suspension before addition of the serum, by using 1 per cent acetic acid in varying volumes, and followed by readjusting the pH to 7.35 by buffer solutions, offered no advantage over the technic described above. (f) Initial freezing of the leukocyte suspension in order to hemolyze the red cells was found to destroy the WBC agglutinability.

6. Tests used in each case. The patients' serum was used fresh or after preservation for several weeks at -20 C.; it was tested without previous treatment, after being heated for 10 and 30 minutes at 56 C., or recombined with guinea pig or fresh human complement. It was brought into contact with donor's leukocytes and the patient's own WBC, and sometimes with leukocytes provided by other patients whose sera contained leukoagglutinin.

Patients' leukocytes were also brought into contact with normal compatible sera. The leukoagglutinin titer was determined by dilution in saline and compatible inactivated human serum. Absorption on WBC and elution of leukoagglutinin were attempted. Fractionation of the sera by the method of Dubert, et al. 27 was carried out to investigate any differences in leukoagglutinating ability of the various fractions. The action of Coombs' antiglobulin on leukocytes was examined employing a technic similar to that used for erythrocytes. Other unsystematized examinations will be described later.

**SEROLOGICAL STUDIES**

*Immunology of Leukocytes*

1. Factors influencing the leukocyte agglutination reaction. (a) The optimum duration of incubation appeared to be 1 hr.; the agglutination reaction begins to be readable after 50 minutes, becomes more intense until it reaches a maximum, then remains unchanged after 1 1/2 to 2 hours. Incubated at 37 C. for more
than 2 hours, the leukocytes form irregular aggregates and exact reading of the reaction is no longer possible.

(b) Although leukocyte agglutination took place at a temperature as low as 4°C and proceeded regularly at 18°C, the optimum temperature for the reaction was found to be 37°C in all cases.

(c) Within a fairly wide zone of pH 6.5 to 9.3, acidification of the suspension with HCl or alkalinization with NaOH did not produce notable modification of the agglutination reaction.

(d) Early in the study it was noted that leukocytes were often more strongly agglutinated after somewhat prolonged conservation of the serum at 4°C. The supposition that this could be accounted for by the presence in fresh serum of an inhibitory factor was subsequently verified. The inhibition was found to be abolished by heating for 30 minutes at 56°C or after preservation at 4°C for several days. Later, heating 2 minutes at 56°C was sometimes found sufficient to destroy the inhibiting effect. It was thought that the complement might be responsible for this effect. However, the addition of fresh human serum or guinea pig complement only diminished the intensity of the agglutination reaction and did not restore the total inhibition. By partially destroying the inhibitor by heat, it was possible to show the role played by the fresh human or guinea pig serum (table 1). During the agglutination reaction, complement is not consumed, as measured by the usual method employing sheep red cells. Furthermore, complement does not seem to be consumed in vivo, since the complement titer is normal in the patients' fresh sera.

(e) Preservation of the leukocyte suspension for 48 hours at 4°C or freezing for 10 minutes at −20°C produced total disappearance of the agglutinability of the cells. In addition, washing the cells three times in normal saline or in compatible human serum considerably diminished or abolished the intensity of the agglutination.

(f) Addition of Coombs' antiglobulin serum, at dilutions of $\frac{1}{2}$, $\frac{1}{5}$, $\frac{1}{10}$, and $\frac{1}{50}$, to washed normal leukocytes previously exposed to the patient's serum, did not augment the intensity of agglutinations. Neither did treatment of the leukocytes.

### Table 1. Influence of an Inhibitory System on the Leukoagglutination Reaction

(Addition of human or guinea pig complement to serum containing leukoagglutinins, heated at 36°C for variable periods.)

<table>
<thead>
<tr>
<th>Leukoagglutinin containing serum heated during</th>
<th>0°</th>
<th>5°</th>
<th>10°</th>
<th>15°</th>
<th>20°</th>
<th>25°</th>
<th>30°</th>
<th>35°</th>
<th>40°</th>
<th>45°</th>
<th>50°</th>
<th>55°</th>
<th>60°</th>
<th>65°</th>
<th>70°</th>
<th>75°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diluted in saline, 1:2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in inactivated human serum, 1:2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in fresh human serum, 1:2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in guinea-pig serum, 1:10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The serum containing leukoagglutinin is capable of provoking leukoagglutination only after destruction or transformation by heat of the inhibitory system. Addition of an equal volume of normal saline or inactivated human serum does not modify the result; addition of fresh human serum or guinea pig complement, on the other hand, results in clear cut inhibition.*
LEUKOAGGLUTININS

Table 2.—Properties of Leukoagglutinins

<table>
<thead>
<tr>
<th><strong>Physicochemical</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermostable</td>
</tr>
<tr>
<td>Destroyed by heating to 65°C for 30 hours</td>
</tr>
<tr>
<td>Preserved by freezing</td>
</tr>
<tr>
<td>A gamma globulin (likely)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Serologic</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum action temperature 37°C.</td>
</tr>
<tr>
<td>Act in a wide zone from pH 6.5 to pH 9.2</td>
</tr>
<tr>
<td>Act in saline and serum media</td>
</tr>
<tr>
<td>Inhibited by a thermolabile serum factor</td>
</tr>
<tr>
<td>Absorbed by leukocytes</td>
</tr>
<tr>
<td>Act on normal and pathologic granulocytes</td>
</tr>
<tr>
<td>Act on normal and pathologic lymphocytes</td>
</tr>
<tr>
<td>Do not act on some leukoblasts</td>
</tr>
<tr>
<td>Partially inhibit phagocytosis of bacteria</td>
</tr>
</tbody>
</table>

cytes by enzymes, such as trypsin, used according to the technic for erythrocytes (Difco 1:250 diluted 1:1000).

(g) Various products were added to the patients’ serum in an attempt to demonstrate the possible intervention of toxic substances to which the patients might be sensitized; these included basic aniline dyes, an emulsion of benzene, chloramphenicol, gold salts, and certain sulfonamides in concentrations from 1:10 to 1:1000. In the one case (no. 1) which manifested agranulocytosis attributed to pyramidon sensitization, addition of pyramidon to the serum obtained during the crisis did not increase the agglutination of normal leukocytes by the serum; neither did addition of pyramidon cause the reappearance of the agglutination reaction in serum obtained thirty days after the agranulocytic crisis.

2. Characteristics of the leukoagglutinin (Table 2). (a) The clumps of agglutinated cells are difficult to examine cytologically because of their density; the cells are shrunken, they are difficult to smear, and they do not take the stain well. Nevertheless, one can distinguish the mononuclear cells from the granulocytes. Among the rare free elements as well, which are more easily stained and identified, the usual ratio of mononuclear cells to granulocytes is found. The leukoagglutination reaction appears to involve both cell types nonpreferentially. The leukocytes of the leukemic patients studied reacted quite variably. In fifteen cases of chronic myeloid leukemia the cells were strongly agglutinated by all twelve leukoagglutinin-containing sera that were tested. In five cases of chronic lymphoid leukemia the white cells were likewise agglutinated by the nine sera tested, but in a more irregular manner. In fifteen cases of acute leukemia the results were even more inconstant when tested against fourteen sera. Certain varieties of undifferenstiated leukemic cells appeared not to be agglutinated at all by the sera containing agglutinin.

(b) Several experiments were conducted in order to determine the percentage of positive results obtained with the same leukoagglutinin-containing sera in the presence of fifty varieties of leukocytes provided by normal donors. In one
of these experiments, fifteen sera were tested; twelve agglutinated all types of leukocytes without distinction and four sera (nos. 5, 10, 11, 16) gave inconsistent results, agglutinating from 95 to 90 per cent of the leukocyte types. We could not classify these results.

(c) The phagocytic function of polymuclear cells tested with three of the leukoagglutinin-containing sera was partially inhibited, thus confirming the findings of Hickie. The test was carried out as follows: after formation of the clumps of leukocytes under standard conditions, a drop of staphylococcus culture was added (containing about 500,000 organisms per cu. mm.). After incubation at 37 C. for 30 min., smears were made of the clumps. The number of cells which had ingested staphylococci was then compared with the number which had not.

(d) The leukoagglutinating substance is thermostable; it retains its properties after heating for 30 minutes at 56 C. and for 10 minutes at 65 C., but after heating for 30 minutes at 65 C. the leukoagglutinating property disappears. Freezing at -20 C. for several months produces little or no alteration in its potency.

(e) Passage through a Seitz filter or absorption by barium sulfate (using 0.1 Gm. per cc. of serum, left in contact for 15 min. at 37 C. and followed by centrifugation) does not alter the leukoagglutinin.

(f) One cc. of the inactivated serum, with a 1:28,000 solution of merthiolate added, was mixed with 0.25 cc. of a suspension of normal leukocytes which had previously been washed three times in saline. The mixture was left at 37 C. for 3 hours, then at 4 C. for 18 hours. After centrifugation the supernatant serum was recovered, and after two or three passages on normal leukocytes under the same conditions, the remaining serum was tested for its ability to agglutinate leukocytes. In three cases (nos. 3, 6, 9) absorption was complete. The necessity of using previously washed leukocytes perhaps accounts for the slowness of the absorption. After contact for 2 hours at 37 C. with the normal leukocytes in the same proportions, the serum was replaced by an equal volume of normal saline and the mixture heated for 10 minutes at 56 C. In two cases (nos. 6 and 14) the eluted saline was capable of strongly agglutinating a new suspension of leukocytes; in the other cases the reading of the test was equivocal.

(g) Sera were fractionated and the various fractions, after being brought to their initial volume, were tested against normal leukocytes. Analogous fractionations were carried out with normal sera. The phenomenon of leukoagglutination was observed exclusively in the globulin fraction. Massive agglutination was observed even at high dilutions using the globulin fraction provided by the pathologic sera, whereas the undiluted serum globulins of the control showed less definite agglutination and none at all at dilutions greater than 1:2.

(h) Electrophoresis of sixteen sera, following the technic of Antweiler, revealed an elevation of the gamma globulin fraction in all the sera tested. In some cases the augmentation was considerable, up to 46 per cent. No consistent modification of the other fractions was noted (table 3).

(i) Two cc. of the sera of four patients and of four controls was placed in contact with a definite number of leukocytes and counts were made after 1 hour, 2 hours, 5 hours, and 18 hours. No significant diminution in the number of leukocytes resulted; the formation of clumps of leukocytes, however, makes the count very inexact. The smears did not present morphologic evidence of lysis. However,
## Table 3.—Electrophoretic Fractionation of Sera with Leukoagglutinin

(Antweiller’s Technic)

<table>
<thead>
<tr>
<th>Case</th>
<th>Total proteins (Gm. %)</th>
<th>Albumin (%)</th>
<th>α1 (%)</th>
<th>α2 (%)</th>
<th>β (%)</th>
<th>γ globulin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>60-75</td>
<td>60</td>
<td>3.5</td>
<td>4.5</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>72.3</td>
<td>51.8</td>
<td>2.6</td>
<td>4.2</td>
<td>14.1</td>
<td>27.1</td>
</tr>
<tr>
<td>3</td>
<td>85.0</td>
<td>50.3</td>
<td>1.0</td>
<td>8.4</td>
<td>38.8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>65.9</td>
<td>47.3</td>
<td>4.4</td>
<td>4.5</td>
<td>13.3</td>
<td>30.4</td>
</tr>
<tr>
<td>5</td>
<td>71.4</td>
<td>46.4</td>
<td>1.6</td>
<td>10.2</td>
<td>41.8</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>77.6</td>
<td>31.1</td>
<td>2.8</td>
<td>3.6</td>
<td>13.9</td>
<td>48.6</td>
</tr>
<tr>
<td>7</td>
<td>88.5</td>
<td>7.5</td>
<td>8.7</td>
<td>13.0</td>
<td>27.2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>63.5</td>
<td>4.7</td>
<td>15.9</td>
<td>15.3</td>
<td>30.4</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>64.8</td>
<td>3.8</td>
<td>6.8</td>
<td>12.4</td>
<td>45.1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>55.8</td>
<td>4.8</td>
<td>8.6</td>
<td>11.7</td>
<td>37.8</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>81.3</td>
<td>2.7</td>
<td>6.5</td>
<td>16.1</td>
<td>42.1</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>76.3</td>
<td>4.8</td>
<td>7.0</td>
<td>8.2</td>
<td>32.6</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>75.6</td>
<td>2.0</td>
<td>4.1</td>
<td>7.6</td>
<td>46.9</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>85.6</td>
<td>2.1</td>
<td>6.0</td>
<td>12.3</td>
<td>39.0</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>60.0</td>
<td>3.6</td>
<td>15.3</td>
<td>12.9</td>
<td>31.9</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>74.3</td>
<td>18.5</td>
<td>3.8</td>
<td>9.9</td>
<td>67.7</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>69.5</td>
<td>3.5</td>
<td>4.8</td>
<td>10.0</td>
<td>35.4</td>
<td></td>
</tr>
</tbody>
</table>

## Table 4.—Categories 1, 2, and 3

<table>
<thead>
<tr>
<th>Case</th>
<th>Clinical findings</th>
<th>Hematology</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>symptoms</td>
<td>treatment</td>
<td>RBC (mill.)</td>
</tr>
<tr>
<td>1*</td>
<td>Agranulocytosis due to pyramidon</td>
<td>0</td>
<td>3.7</td>
</tr>
<tr>
<td>2†</td>
<td>Hypochromic anemia following congenital angiomatosis</td>
<td>305 transfusions</td>
<td>2.6</td>
</tr>
<tr>
<td>3‡</td>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>Transfusions</td>
<td>2.4</td>
</tr>
<tr>
<td>4‡</td>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>Transfusions</td>
<td>2.9</td>
</tr>
<tr>
<td>5‡</td>
<td>Atypical paroxysmal nocturnal hemoglobinuria</td>
<td>Transfusions</td>
<td>1.4</td>
</tr>
</tbody>
</table>

* Category 1: allergic agranulocytosis.
† Category 2: patient with multiple transfusions.
‡ Category 3: paroxysmal nocturnal hemoglobinuria.
serum no. 13 induced signs of premature death of the normal white cells when examined with the phase microscope by Bessis.26

(j) Two sera containing leukoagglutinin (provided by cases 6 and 8) were injected into two patients. One patient with chronic lymphoid leukemia received 150 cc. of serum. A marked fall of the leukocyte count from 36,000 to 5400 per cu. mm. was observed immediately and lasted twelve hours, while normal sera injected as a control into the same patient brought about no such decrease. The second patient, who had an unspecified malignancy, had an original white count of 8500 and exhibited a briefer and less marked fall.

3. Studies of the pathologic (patients') leukocytes. With many variations of technic, we have not yet succeeded in demonstrating the phenomenon of agglutination, using fresh or inactivated serum, with homologous leukocytes. Exposing patients' leukocytes to normal sera under the usual conditions showed in three cases (nos. 6, 9, 10) feeble agglutination with the formation of clumps less compact and morphologically different from the usual picture in a positive reaction. In these three cases, malignant blood diseases were diagnosed. It is possible that these atypical aggregations are equivalent to the "leukergie" described by Fleck27 and Tischendorf and Fritzell.28 Addition of Coombs' antiglobulin serum to pathologic leukocytes after three washings in saline did not produce agglutination. Previous treatment of the leukocytes with trypsin had no effect upon the agglutination reaction.

Immunology of Erythrocytes

1. No correlation of the ABO system and other blood systems with the presence or absence of leukoagglutinins was noted.

2. The possibility of atypical agglutinations was systematically investigated upon a series of selected donors including all known antigens; tests were carried out in saline and in bovine albumin in the presence of Coombs' antiglobulin and by the method of trypsinized red cells. It was possible to demonstrate irregular agglutinins (anti-Cw and anti-C + D) in the sera of two patients who had received

### Table 5.—Category 4: Symptomatic Pancytopenia

<table>
<thead>
<tr>
<th>Case</th>
<th>Clinical findings</th>
<th>Hematology</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>symptoms</td>
<td>treatment</td>
<td>blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RBC (mill.)</td>
</tr>
<tr>
<td>6</td>
<td>Acute leukemia</td>
<td>Transfusions, cortisone</td>
<td>1.3</td>
</tr>
<tr>
<td>7</td>
<td>Acute leukemia</td>
<td>Transfusions, cortisone</td>
<td>2.4</td>
</tr>
<tr>
<td>8</td>
<td>Hodgkin's disease</td>
<td>Transfusions</td>
<td>3.6</td>
</tr>
<tr>
<td>9</td>
<td>Lymphosarcoma</td>
<td>Transfusions, cortisone</td>
<td>2.7</td>
</tr>
<tr>
<td>10</td>
<td>Lymphosarcoma</td>
<td>Transfusions, splenectomy</td>
<td>2.6</td>
</tr>
</tbody>
</table>
LEUKOAGGLUTININS

multiple transfusions. These sera were found to be capable of agglutinating leukocytes of donors belonging to groups C— and cde/cde. Likewise, anti-D sera similarly used were incapable of producing leukoagglutination of leukocytes provided by D-positive donors. These observations permit us to affirm that the leukoagglutinin is entirely distinct from the specific antierythrocytic antibodies.

3. Tests for warm panantibodies fixed on the patients’ own red cells or free in the plasma (albumin, Coombs’, and trypsin tests) were in most cases negative. However, in one case (no. 9) the direct Coombs’ test was temporarily positive. In four cases (nos. 9, 12, 16, 17) agglutination of trypsinized isoerythrocytes suspended in serum was observed. Eight sera containing warm and cold auto-antibodies (from patients with hemolytic anemias) were tested without showing any leukoagglutination, thus demonstrating the independence of the two phenomena.

4. The life span of transfused red cells was studied in two cases by the technic of Ashby.29 In case no. 18, one of those in the category of idiopathic pancytopenias, definitely accelerated destruction was demonstrated. Sixty days after transfusion, the surviving red cells had fallen to 20 per cent instead of 50 per cent as expected according to the theoretic curve, and in eighty-six days only 8 per cent remained. The curve of disappearance was exponential in type, suggesting the intervention of an extrinsic factor (fig. 4). In any case, the frequency of transfusions required to maintain this patient’s red cell count in the region of 3,500,000 per cu. mm. suggested an excessive rate of destruction. In case 3, a patient with PNH, the rate of transfused red cell destruction approximated the theoretic curve (fig. 4).

Table 6.—Category 5: Chronic Idiopathic Pancytopenia

<table>
<thead>
<tr>
<th>Case</th>
<th>Clinical findings</th>
<th>Hematology</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RBC (mill.)</td>
<td>plat. (thous.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Asthenia, ulcerations of the mouth + 1 year</td>
<td>Transfusion, cortisone = 0</td>
<td>2.4</td>
</tr>
<tr>
<td>12</td>
<td>Asthenia, splenomegaly + 1 year</td>
<td>Transfusion, splenectomy</td>
<td>3.0</td>
</tr>
<tr>
<td>13</td>
<td>Asthenia, chronic pallor</td>
<td>Transfusion, cortisone = 0</td>
<td>3.9</td>
</tr>
<tr>
<td>14</td>
<td>Asthenia, pallor hemorrhagic syndrome</td>
<td>Transfusion, ACTH = 0</td>
<td>2.4</td>
</tr>
<tr>
<td>15</td>
<td>Asthenia</td>
<td>Transfusion</td>
<td>3.1</td>
</tr>
<tr>
<td>16</td>
<td>Asthenia, hemor- rhagic syndrome, splenomegaly</td>
<td>Transfusion, splenectomy = 0</td>
<td>2.0</td>
</tr>
<tr>
<td>17</td>
<td>Felty’s syndrome cirrhosis + 1 year</td>
<td>Transfusion, ACTH = ± 12</td>
<td>3.2</td>
</tr>
<tr>
<td>18</td>
<td>Asthenia, chronic pallor</td>
<td>Transfusion, cortisone = ± 12</td>
<td>1.7</td>
</tr>
<tr>
<td>19</td>
<td>Asthenia, pallor, hepatic disorders</td>
<td>Transfusion, cortisone</td>
<td>3.1</td>
</tr>
</tbody>
</table>

For personal use only. On August 16, 2017, by guest. www.bloodjournal.org
Immunology of Platelets

Examination of the sera in question for the presence of thrombocyte agglutinins, using a personal technic and the technics of Stefanini or Harrington, produced consistently negative results. In only one case (no. 16) was the test positive, and in this case splenectomy abolished the thrombocyte agglutination reaction.

In conclusion, immunologic studies of the sera of nineteen patients permit us to affirm the existence, in certain pathologic conditions, of a serum substance capable of agglutinating normal leukocytes. The property is most marked in previously inactivated serum. It is independent of the red cell agglutinins which may coexist. The agglutinating substance is found in the gamma globulin fraction of the serum. It has been impossible to demonstrate agglutination of the patients' own leukocytes by the corresponding sera. Coombs' antiglobulin produced no effect upon the pathologic (patients') leukocytes. The technic used in studying the immunology of leukocytes must be widely extended before these results can be fully interpreted and definite conclusions drawn.

DISCUSSION

Serologic Aspects

Validity of the leukoagglutination reaction

The sera used in the study were taken from more than two thousand normal subjects, five hundred patients with various diseases, and one hundred two patients with leukopenia. Leukoagglutination was found only in the nineteen cases described. In these nineteen cases, it was consistently present in at least fifty examinations of each.

Causes of false positive reactions. 1. Rouleaux formation of leukocytes. In this phenomenon, described by Bond and Tullis, the leukocytes are arranged in rolls similar to red cell rouleaux.

2. Leukergia. The leukergic phenomenon described by Fleck is the agglutination of leukocytes in their own and in normal citrated plasma.

3. Erythrophagocytosis. Leukocytes have a tendency to surround and phagocytose clumped red cells. For this reason, our tests have always matched serum against leukocytes of the same or compatible ABO groups. In case of incompatibility, the red cells are agglutinated and after hemolysis by the addition of acetic acid, the leukocytes become enmeshed in the stroma which remains.

4. Contamination of serum. Leukocytes surrounding a culture of bacteria in a contaminated serum may be confused with an agglutinate. This possibility was eliminated by using fresh sera or sera frozen at -20 C.

5. L.E. phenomenon. Antileukocytic sera, both experimental and human, were shown to be able to induce pictures comparable to those observed with L.E. serum. Some of these sera have phagocytic properties as well as agglutinating properties.

Causes of false negative reactions. 1. Inhibitory system. Sera containing leukoagglutinins were incapable of producing the agglutination described unless they were first heated for 2 to 10 minutes at 56 C. In a single case with stronger leukoagglutinin, the inhibition was only partial before heating. Our findings suggest that a normal thermolabile system is involved, since the inhibitory factor is found either in fresh normal or pathologic serum containing leukoagglutinin, and in fresh guinea pig serum (table 1). It is known that complement possesses an inhibitory action on erythrocyte agglutination. This inhibitory action seems stronger in regard to the leukoagglutination than to the red cell agglutination. The
LEUKOAGGLUTININS

Complement was shown by delicate titer technics\(^3\) to be slightly consumed during erythrocyte agglutination. By rough technic we have not yet observed such consumption during leukoagglutination.

2. Leukolysis. Disappearance of the cell bodies has never been observed, even with sera of high leukoagglutinin titer. But partial leukolysis may be responsible for a prozone phenomenon sometimes observed.

It is thus clear that the causes of error can easily be eliminated. True agglutination such as was observed in every instance consisted of compact, dense, tight cellular masses analogous to those seen in erythrocyte agglutination.

**Immunologic mechanism of leukocyte agglutination**

We believe the phenomenon to be immunologic. Both human and animal leukocytes have been shown to be antigenic,\(^{35-38}\) and there is even a specificity of antigenic reaction for neutrophils\(^{39-42}\) and lymphocytes.\(^{43-46}\) Thus immune leukocytic heteroantibodies can be prepared and demonstrated. Leukocytic isoantibodies, on the other hand, are not yet as clearly understood. Doan,\(^6\) using a vital staining technic, described leukocyte blood groups and classified all individuals into two groups: group A (40 per cent), in which leukocytes are compatible with all plasma, and group Z (the remaining 60 per cent), in which leukocytes are incompatible with some or all plasma. Wichels and Lampe\(^6\) presented suggestive evidence of there being leukocyte agglutinogens A and B in two cases of myeloid leukemia and in a case of lymphocytic leukemia. Tullis\(^4\) absorbed agglutinins anti-A and anti-B on white cells and suggested that leukocytes are similar to erythrocytes and carry antigens A and B.

In our study, the following arguments are in favor of the immunologic nature of the leukoagglutination phenomenon: (1) Gamma globulins were shown by electrophoresis to be considerably increased in all cases, and leukoagglutinins were shown by fractionation of serum to be included in the gamma globulin fraction. (2) By the absorption-elution test, leukoagglutinins can be absorbed by normal leukocytes. Elution of the leukoagglutinin from agglutinated leukocytes has been accomplished in the two cases in which leukoagglutination was particularly strong. (3) Passive transfer of leukoagglutinin has been possible in the two cases in which it was attempted. In these two cases the injection of serum containing the leukoagglutinin produced an immediate profound drop in the leukocyte count of the recipient; whereas, injection of normal serum provoked no such response.

**Types of antileukocytic antibodies**

If we accept the fact that we are dealing with a specific antibody against leukocytes, we must attempt to determine its nature, making analogy with the antierthrocytic antibodies. We are not justified in drawing definite conclusions from our facts, but some speculations concerning the identity of the observed leukoagglutinin seems in order. In the first place, it does not appear likely that it is a natural antibody, since leukoagglutination has been encountered only in pathologic sera. Several series (one in collaboration with van Loghem and Goudsmit) crossmatching numerous normal sera against normal leukocytes have not produced clear evidence of the existence of natural antibodies. However,
the number of subjects tested is insufficient to permit an absolute exclusion of this possibility. In favor of the hypothesis that we are dealing with immune antibodies we cite the fact that all patients demonstrating antileukocytic antibodies had received transfusions, which might have resulted in sensitization. Unfortunately, these transfusions were given before the test was performed. However, the pathologic sera tested the same day against fifty varieties of normal leukocytes taken at random did not show a constant percentage of positive and negative results. The 0 to 5 per cent negative results were not constant or could not be repeated. These imprecise results are perhaps due to the fact that the technic is not yet perfected, and that the leukocytes can be used only for a few hours after drawing. However, if it is a group isoantibody, the large number of positive reactions is in favor of a sensitization against an extremely common antigen or many antigens. We have not had the opportunity of observing the relation between pregnancy and the appearance of leukoagglutinins.

We must now consider the hypothesis of autoimmunization of the individual against his own leukocytes as in certain hemolytic anemias with autoantibodies. In every case but one (no. 2) leukopenia was marked, suggesting a relationship between leukopenia and the leukoagglutination. In order to test this hypothesis we have tried numerous times and with different technics to produce agglutination by a pathologic serum of its own leukocytes, but have been unable to demonstrate it. Bessis has observed autophagocytosis and phagocytosis of pathologic leukocytes by normal leukocytes. Similarly, autophagocytosis of pathologic leukocytes occurs to some degree. Fixation on leukocytes of an opsonin thus seems to be demonstrated. Furthermore, we have added Coombs' antiglobulin to washed pathologic leukocytes and to normal leukocytes after contact with the pathologic sera, without observing agglutination. The existence of blocking agglutinins has not been verified. The coexistence of leukopenia with leukoagglutinins and the Bessis experiment are the best arguments for the existence of leukocytic autoantibodies.

Nevertheless, this distinction between group isoantibodies and autoantibodies is perhaps not so absolute, especially since Weiner, et al. have recently demonstrated (in the case of antigen e of the Rh system) that an organism can sensitize itself against a group antigen carried by its own cells. It is not impossible that a similar mechanism operates for leukocytes.

Finally, the concept of an antileukocytic antibody which necessitates the presence of an allergen to operate can be considered only in one case (no. 1). However, the action of pyramidon, evident in vivo, could not be demonstrated in vitro.

In conclusion, it seems possible to assert that there exists in certain pathologic states, a thermostable serum leukoagglutinin inhibited by a thermolabile system. This leukoagglutinin is to all appearances an antibody of which the type—isoantibody, autoantibody, or allergic antibody—remains to be determined.

Analysis of Clinical Material

Clinically, the patients whose sera contained a leukoagglutinin may be divided into five categories: (1) a single case of agranulocytosis with pyramidon (aminopyrine) hypersensitivity; (2) one patient who has received three hundred five transfusions; (3) three cases of paroxysmal nocturnal hemoglobinuria (Marchia-
fava-Micheli syndrome); (4) five cases of malignant hemopathies; and (5) nine cases of aplastic or hypoplastic anemia. This clinical classification, admittedly arbitrary, nevertheless permits us to correlate the serologic findings.

**Category 1.** In the single case of hypersensitivity to pyridon, administration of the drug was followed by a veritable crisis in the granulocyte series. Within a few days after stopping the drug, the granulocytopenia disappeared and was replaced by leukocytosis with a predominance of polymuclear cells. The leukoagglutinating property of the serum, present concomitantly with the granulocytopenia, likewise disappeared. Addition of pyridon to the serum in vitro did not induce reappearance of the response. Intradermal testing with the patient's serum, pyridon added, produced no local reaction. The severity of the initial granulocytopenia made it inadvisable to attempt readministration of the drug.

**Category 2.** The second category is set apart because this patient (no. 2) had received a particularly high number of transfusions; the underlying pathology was hemorrhagic angiosatosis with hypochromia and disturbed iron metabolism. Very severe transfusion reactions began to appear after one hundred and fifty transfusions. This could not be explained on the basis of ordinary red cell group incompatibility, nor could antiplatelet antibodies be demonstrated. Intolerance to plasma analogous to that described by Dameshek, et al. was suspected. Ten washings of the cells were necessary to eliminate the reactions. It was therefore thought that the transfusion reactions could be the result of incompatibility of the transfused leukocytes with the leukoagglutinin shown to be present in this patient's serum. It should be noted that the other patients whose sera contained leukoagglutinin underwent repeated transfusions without any particular reaction. This is the only case in our series which did not show leukopenia; the patient had been splenectomized. It remains to be definitely established that this case represents prior sensitization by multiple transfusions. Further studies are in progress.

**Category 3.** In all three cases of paroxysmal nocturnal hemoglobinuria (PNH) which we were able to examine, leukoagglutinin was demonstrated. The frequency of leukopenia in this condition is recognized. Nelson and Bruce have even described a case evolving toward an aplastic bone marrow. Two of our cases presented an extreme leukopenia with less than 2000 leukocytes per cu. mm.; the white cell count of the third patient varied between 3000 and 4000 per cu. mm. In all three cases there was also a moderate thrombocytopenia. The bone marrow showed, in addition to a strong erythroid reaction, a relative arrest of maturation at the metamyelocyte stage. It is perhaps premature to attempt an interpretation of these observations; they may simply represent sensitization of certain varieties of leukocytes by the twelve, eighteen, and fifty-eight transfusions which our patients had respectively received. However, the constancy of leukopenia in PNH and the concomitant presence of leukoagglutinins in our three cases suggest a more than coincidental connection between these two facts. According to the current conception of the disease, as recently set forth by Crosby, PNH is not exclusively a disease of red cells, but represents the simultaneous disturbance of all three blood cell series due to "permanent alteration of those parts of the reticuloendothelial system which produce the proteins of the stromata of blood cells." Crosby supposes that this alteration may represent "a perversion of the immune response to viral or plasmodial infection." It seems reasonable to suggest that the substance which attacks the divers bone marrow elements may well be related to the leukoagglutinin we have observed.

**Category 4.** In the fourth category we have grouped five patients with malignant hemopathies: namely, two cases of acute aleukemic leukemia, one case of Hodgkin's Disease, one case of lymphosarcoma, and one case of reticulosarcoma. Here too one cannot exclude the possibility of previous sensitization by the transfusions that all our patients had received before their sera were tested for leukoagglutination. In one case (no. 6), however, the leukoagglutinin was noted in the serum soon after the first transfusion and before the expected time for antibody formation had elapsed. Leukoagglutinins were demonstrated in each case coincidentally with profound leukopenia. Correspondence of the leukoagglutinin titer with the degree of leukopenia was noted, but was not absolute. In one case (no. 9)
cortisone brought about amelioration of the leukopenia and a corresponding decrease in the leukoagglutinin titer. This change was abolished as soon as administration of cortisone was halted. A similar result was noted in case no. 10 after splenectomy, the leucocyte count falling and the leukoagglutinin titer increasing two months after operation. One case (no. 6) showed no parallelism between the leucocyte count and the leukoagglutinin titer. This was a case of acute leukemia which remained aleukemic for three months. At the time of invasion of the peripheral blood by leucoblasts, the serum leukoagglutinin persisted in undiminished titer. The fact that we are here dealing with leukemic leukocytes may explain the dissociation. It must be emphasized that leukoagglutinin is not always present in the serum of patients with these types of hemopathy. We found no leukoagglutinin in the sera of fifteen other patients with chronic myeloid leukemia, five with chronic lymphoid leukemia, six with acute leukemia, and eight with Hodgkin's disease. However, none of these latter patients was leukopenic.

Category 5. The last category of patients consists of eight with chronic idiopathic pancytopenia occurring in aplastic or hypoplastic anemia. These patients were aged 30 to 75 years and presented the usual clinical history: insidious onset marked by weakness and pallor, with no previous history of exposure to poisons or infections. Treatment and medications had been banal and not noted for the likelihood of producing leukopenia. Physical signs were minimal; there was moderate splenomegaly in two cases but otherwise no splenomegaly, hepatomegaly, or lymphadenopathy. The majority of cases did not show clinical signs of hyperhemolysis, but in three cases (nos. 12, 16, 17) icterus or subicterus was noted coincidentally with the appearance of splenomegaly. In case no. 6 there were large numbers of erythroblasts in the peripheral blood. In these three cases an anti red cell panantibody was demonstrated by the trypsinized red cell agglutination test.

Examination of the blood revealed pancytopenia; normochromic, normocytic anemia with 2 to 3 million red cells per cu. mm.; moderate but constant thrombocytopenia (100,000 to 150,000 platelets per cu. mm.); and very marked leukopenia, with between 700 and 3000 leucocytes per cu. mm. There was absolute and relative neutropenia, with between 20 and 40 per cent of polyncuculars and sometimes a shift toward the left. In most cases the marrow was hypoplastic, but in two cases the marrow was normal or even hyperplastic; in these cases an erythroid reaction was present, masking the hypoplasia of the myeloid series. In case no. 15 the percentage of elements was normal, while in cases 12, 13, 16, and 18, arrest of development at the myelocyte stage was observed. In one case (no. 18) abnormal cells were found, classified by some observers as monocytes, by others as abnormal myeloid elements.

The course of the disease was chronic, extending over several years. Hemorrhagic complications due to the thrombocytopenia were noted, but the most common complications were those attributable to the leukopenia and the consequent increased susceptibility to infection. In two patients (nos. 16, 17), who had splenomegaly, cirrhosis developed. Divers methods of treatment were tried with disappointing results; these included vitamin B\textsuperscript{12}, liver extract, and iron. Transfusions permitted maintenance of the red cells at about 3,000,000 per cu. mm., but the volume transfused did not always correspond to the amelioration observed; the leukopenia and thrombocytopenia were not affected. Splenectomy was performed in two cases. In case 12 the patient died after the intervention in a hemorrhagic diathesis. In case 16 the operation produced definite amelioration. That is, less frequent transfusions were required, the hemorrhagic tendency disappeared, and the leucocyte count returned to normal. After two months, however, the leukopenia reappeared. Cortisone, 200 mg. per day or ACTH, 100 mg. per day, was administered for thirty days in cases 11, 13, 14, 17, 18, and 19. During the treatment a moderate elevation of the white cell count was noted, which disappeared as soon as the treatment was discontinued. Hansen\textsuperscript{4} has been able to obtain considerable amelioration in one similar case treated with cortisone, and has proposed an immunologic mechanism to explain the improvement. Death supervened in three cases. In case 12 it immediately followed splenectomy, in case 17 it was due to cirrhosis, and in case 11 to infection. Postmortem studies were carried out in two cases, but failed to elucidate further.
From the point of view of pathologic classification, these cases are poorly defined. They do not fit the definition of Schultz of “acute agranulocytosis”; neither do they fit the criteria for aplasias of infectious, nutritional, or endocrine origin. No exposure to toxins or to substances known to provoke sensitization was found. There was nothing to suggest myelofibrosis. The fact that the bone marrow was almost always hypoplastic makes it unlikely that the role of the spleen was primary. Finally, the duration of the malady and the consistent blood picture differentiates these cases from malignant hemopathies with initial aplasia. All of the cases of this category thus seem to fall into the loose classification of aplastic (or hypoplastic) anemias, or “chronic pancytopenias.”

Various other patients with comparable blood pictures were studied, including twenty-one cases of aplasia associated with proved intoxications, ten due to benzol, fourteen cases of bone marrow aplasia with splenomegaly, nine cases of leukopenia associated with infection, twelve cases associated with cirrhosis, two cases of periodic neutropenia, one case of neutropenia concurrent with Besnier-Boeck-Schaumann Disease (sarcoidosis), and twenty-two cases of leukopenia or neutropenia of undetermined origin. Some of these patients presented clinical and hematologic pictures very similar to those described above, but none demonstrated the leukoagglutination phenomenon.

**Correlation of Clinical and Serologic Findings**

Of the two thousand normal and five hundred patients’ sera which were studied, only nineteen sera possessed the property of producing leukoagglutination. Setting aside the single patient who had received multiple transfusions (no. 2), it is notable that the presence of leukopenia with neutropenia was the sole clinical feature common to all eighteen of the others. This fact seems to us more than coincidental. It seems reasonable to propose that the leukoagglutinating substance may play a determining role in the development of the leukopenia in these cases. In our discussion we will also take up the question of the mechanism whereby the simultaneous disturbance of all blood cell types is brought about. Finally, by analogy with the hemolytic anemias, we will present a tentative classification of the several syndromes observed.

**Proposed physiopathologic role of the leukoagglutinating substance.** There are several arguments in favor of the direct intervention of the leukoagglutinin in producing the characteristic blood picture referred to above. In the patient with pyridon hypersensitivity, the leukoagglutinin was present only as long as there was granulocytopenia. In all the other cases, the leukoagglutinin titer followed more or less the fluctuations in the white cell count, albeit imperfectly, and here too the agglutinating substance was present only in sera of patients who are or were leukopenic. It seems that the test may remain positive after the leukemic phase has passed, as in the case for the parantibodies in the hemolytic anemias. Finally, in two cases in which transfusion with blood shown to contain leukoagglutinin was carried out, the recipient developed marked leukopenia. Kissmeyer-Nielsen has reported the same phenomenon.

These observations lead to the conclusion that the serum substance which manifests its presence by the agglutination reaction probably has a noxious effect upon the patients’ own leukocytes. In the absence of a good test for lysis...
of circulating leukocytes, however, it is not possible to state whether the site of action is peripheral or whether the bone marrow is directly attacked. If the site of leukocyte destruction is peripheral, it might be due to an intravascular lysis or to lysis or phagocytosis in certain specific organs. The role of the spleen here seems less important than in the red cell series; instead, the lung may well be of predominant importance.

*Simultaneous disturbance of all three cell types.* The fact that in most of our patients leukopenia, anemia, and thrombocytopenia were simultaneously observed is difficult to explain. In the majority of cases, we were not able to demonstrate antiplatelet or anti red cell antibodies which might have explained the anemia and thrombocytopenia. In four cases, however (nos. 9, 12, 16, 17), we did show the presence of a panantibody active only against trypsinized red cells. In only one case was a thromboagglutinin demonstrated, and this disappeared after splenectomy; the operation also produced clinical improvement. The disturbance in most cases was not simply peripheral; the bone marrow was usually hypoplastic, with occasional arrest of development at variably early stages. We did not find any intense myeloid reaction to parallel the erythroblastic response seen in hemolytic anemia. According to Moeschlin, the hypoplasia may be attributed to bone marrow exhaustion. In order to explain the simultaneous disturbance of all three cell types on the basis of the presence of specific agglutinating antibodies, it is necessary to suppose either that the technics for their detection are not sufficiently delicate, or that the antileukocyte substance has a polyvalent action upon a protein substrate common to all three series, or finally that the thrombocytopenia and anemia are produced by some other mechanism as yet unknown.

*Comparison of acquired hemolytic anemias with pancytopenias.* We have found it expedient to draw parallels between the findings in acquired hemolytic anemias and the cases of pancytopenia studied. The acquired hemolytic anemias (those due to factors extrinsic to the cell itself) have been attributed to (1) the action of bacteria or bacterial toxins, (2) exogenous poisons, (3) the intervention of an immunologic mechanism, and (4) "hypersplenism." The third group includes hemolytic anemias due to isosensitization, the anemias with autoantibody or panantibodies, and perhaps certain anemias of allergic origin (table 7).

In the leukocyte series, the analogous phenomena have been investigated.

<table>
<thead>
<tr>
<th>Autoimmunization proper</th>
<th>Allergic autoimmunization</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC Acquired hemolytic anemia with cold or warm autoagglutinins or hemolysins idiopathic or symptomatic</td>
<td>Favism? Bagdad anemia?</td>
</tr>
<tr>
<td>Paroxysmal cold hemoglobinuria</td>
<td>Thrombocytopenic purpura due to sedormid, quinine, or quinidine</td>
</tr>
<tr>
<td>Platelets Thrombocytopenic purpura with thromboagglutinins or lysins</td>
<td>Agranulocytosis due to pyridon</td>
</tr>
<tr>
<td>Leukocytes Leukopenia with leuкоagglutinins or lysins idiopathic or symptomatic*</td>
<td></td>
</tr>
</tbody>
</table>

* Always associated with anemia and thrombocytopenia.
LEUKOAGGLUTININS

Leukotoxins of parasitic or bacterial origin have been demonstrated in vitro by their agglutinating or lytic action. The ability of certain poisons to produce leukopenia is well known. Squier and Lee have presented evidence for the immunologic mechanism of some allergic leukopenia. By analogy with the immunologic hemolytic anemias, one can consider that the leukoagglutination and leukolysis observed in vitro may reflect increased leukocyte destruction in vivo. The substances found to produce these effects in vitro have the characteristics of antibodies. It is therefore suggested that like red cells, the leukocytes may sometimes become antigenic.

The chief objection to this interpretation with reference to our cases has been the failure of the leukoagglutinin-containing sera to have effect upon the patients' own leukocytes. However, the phenomenon of autophagocytosis observed by Bessis suggests one possible explanation for this. That is, the substance which by our technic manifests its presence by agglutination, may be the same as, or one related to, that which produces autophagocytosis.

As in the hemolytic anemias, the underlying cause of such autoimmunization, the nature and character of the antigen, and the mode and site of antibody formation all remain to be determined.

According to this explanation, our cases of the fifth category, in which no infection, intoxication, or other pathologic processes was present would correspond to the idiopathic acquired hemolytic anemias with autoantibodies and could be called "chronic idiopathic pancytopenias with leukoagglutinin." The cases of category 4 (the malignant hemopathies with leukoagglutinin) would correspond to the symptomatic acquired hemolytic anemias and could be called "symptomatic pancytopenias with leukoagglutinin." The immunologic leukopenias, hypoplasias, and aplasias would then complete the outline of hematologic conditions attributed to autoimmunization, summarized in table 7. In the cases of categories 2 (multiple transfusions) and 3 (paroxysmal nocturnal hemoglobinuria) the role of the leukoagglutinating substances remain uncertain.

CONCLUSIONS

The immunology of the leukocyte series in man is still being developed and the technics require considerable extension. The leukoagglutination test here described constitutes a simple technic which permits the examination of one serologic manifestation of the different antileukocytic antibodies which may be present in human serum.

The antileukocytic substances which we have shown to accompany the development of idiopathic or symptomatic pancytopenias correspond to the present criteria for autoantibodies, and may play a causative role in these conditions.

Clinically, if one attempts to classify the various causes of leukopenia as a symptom, one can conceive with Dameshek of the existence of leukopenias by (1) bone marrow disturbance (infection, deprivation of vital foodstuffs, endocrine malfunction, bone marrow sclerosis or infiltration), (2) disturbed release of formed elements from the bone marrow, or (3) excessive peripheral destruction by a toxic or an immunologic mechanism. The leukoagglutination test may perhaps permit differentiation of the toxic from the immunologic leukopenias.
SUMMARY

1. A simple test for the detection of leukoagglutinating properties of serum is described.
2. A thermolabile serum system which inhibits the leukoagglutination was observed.
3. Sera of about two thousand normal subjects and six hundred patients with various diseases were tested. The sera of nineteen of the patients gave positive reactions.
4. The serologic and physicochemical properties of the leukoagglutinating substance were studied, and evidence is presented in favor of its being an antibody.
5. The role of the leukoagglutinating substance in the development of leukopenia is discussed, as well as the probable type of antibody responsible for the leukoagglutination reaction.

SUMMARIO IN INTERLINGUA

1. Es describite un simple essayo pro deteger proprietates leucoagglutinantiane del sero.
2. Un substantia thermolabile que inhibi le leucoagglutination esseva observate in le sero.
3. Esseva essayate seros ab circa 2000 individuos normal e 600 patientes con varie morbos. Reactiones positive esseva constatat in le seros de 19 del patientes.
4. Le proprietates serologic e physiochimic del substantia leucoagglutinantiane esseva studiate. Es presentate datos que supporta su identification como anticorpore.
5. Es discutite le rolo del substantia leucoagglutinantiane in le disveloppamento de leucopenia, como etiam le typo probable del anticorpore responsabile pro le reaction leucoagglutinantiane.

ADDENDUM

Since the submission of this work we have been able to study twenty-five new leukoagglutinating sera. All the forty-five cases can now be classified as follows: one agranulocytosis due to pyramidon, twenty idiopathic pancytopenia, nine symptomatic pancytopenia, five paroxysmal nocturnal hemoglobinuria, six acquired hemolytic anemia, and three anemia or thrombocytopenia having received multiple transfusions.

CASE REPORTS

Case 1

Agranulocytosis due to pyramidon. In 1953, Mme. M., aged 55, the previously healthy mother of one, was hospitalized because of severe pharyngitis with false membrane, accompanied by considerable cervical adenopathy. Blood count: WBC 9400 with normal differential; RBC 4,260,000. Two weeks after discharge, a sciatic pain on the left side was treated for eight days with a total dosage of 2.50 Gm. dimethyl-amino-phenazone, and 7.50 Gm. pyramidon. The patient returned to the hospital with a temperature of 104 F. and alarming general condition. Cervical adenopathy had reappeared. The blood count showed agranulocytosis: WBC 700 per cu. mm., polymorphonuclear 0 per cent, plasmocytes 13 per cent, lymphocytes 75 per cent, monocytes 12 per cent; RBC 3,700,000. The bone marrow was
hypoplastic. Successive blood counts showed steady regression of the agranulocytosis. Ten days after admission the blood count was normal. The patient was seen again two months later and found to be in good condition.

Case 2

Hypochromic anemia following hemorrhagic angiomatosis. Multiple transfusions. Mr. G., aged 44, was known to have been anemic at the age of 20. At 25 a hemorrhagic syndrome appeared, with one or two abundant epistaxis every month; this persisted for many years. At 32, splenomegaly was first noted and varicose telangiectasis was found, scattered over the face and the edges of the tongue. At this time blood examinations showed only a hypochromic anemia. The bone marrow was hyperplastic, with considerable erythroblast reaction (41 per cent). From 1946 to 1949, the patient received eighty transfusions. Bleeding of the gingivae appeared. One transfusion every three weeks became necessary. Irons and liver extract gave no result; therefore, the patient was splenectomized. This brought about only a brief remission of hemorrhage and of the hypochromic anemia. Transfusions were still necessary. After the one hundred and fiftieth transfusion, each successive administration of blood brought on a severe transfusion reaction. Local radium applications halted the epistaxis for three months. Iron absorption was found to be normal, but serum iron fell below 30 gamma per cent in seven to ten hours. This patient has now received three hundred and five transfusions.

Case 3

Paroxysmal nocturnal hemoglobinuria. Mme. C., the previously healthy mother of two aged 44, presented in 1951 a severe hemolytic syndrome with splenomegaly. Anemia reached 760,000 RBC per cu. mm. and leukopenia was marked from the onset: WBC 2300 per cu. mm., polynuclears 62 per cent, eosinophiles 1 per cent, lymphocytes 35 per cent, monocytes 2 per cent; platelets 55,000. Bone marrow showed a defect in maturation of granulocytes and erythroblasticosis. Nocturnal hemoglobinuria appeared one month after onset of the febrile illness. Positive heat, acid, and Crosby tests were found. Osmotic and mechanical fragilities were normal. Treatment with phenylindanedione gave no results. Transfusions of washed red cells have been given every two weeks since 1951. Anemia persists at present at about 2,500,000 RBC per cu. mm.; leukopenia at about 3000 with 30 to 40 per cent polymorphonuclears. Bone marrow is now hyperplastic. Differential marrow count now shows polymorphonuclears 4.5 per cent, other granular cells 40 per cent, lymphocytes 2.5 per cent, erythroblasts 53 per cent.

Case 4

Paroxysmal nocturnal hemoglobinuria. Since 1947, Mr. B., aged 29, previously well and with a negative family history, presented typical attacks of PNH. Jaundice developed in 1951. Blood count: RBC 2,950,000 per cu. mm.; color index 1.1; WBC 2400 per cu. mm., polynuclear neutrophils 14 per cent, other granular cells 32 per cent, lymphocytes 9 per cent, monocytes 2 per cent, erythroblasts 43 per cent. Incubation at 37 C. showed an extremely marked increase in red cell fragility; acid and Crosby tests were positive. Osmotic and mechanical fragilities were normal. Urobininuria and hemosiderinuria were marked. Transfusions of washed red cells have been given every month. Anemia has averaged 3,500,000 to 4,000,000 RBC per cu. mm., the leucocyte count varying from 3800 to 6000 per cu. mm., with 50 to 60 per cent neutrophils.

Case 5

Atypical paroxysmal nocturnal hemoglobinuria. Mme. V., aged 51, mother of two, with negative family history, presented pallor from childhood. It was not until 1951, however, that chronic hemolytic anemia was discovered during a severe infection. Enlargement of the spleen and liver were noted. Puncture of the liver showed massive pigment deposits. Blood count: RBC 1,480,000 per cu. mm.; color index 1; WBC 3700 per cu. mm., with 63
per cent polymorphonuclear neutrophils, reticulocytes 15 per cent. The bone marrow was hyperplastic. Indirect serum bilirubin and stereobilin were increased. Hemoglobinuria was present intermittently, but there was no hemosiderinuria. Serum iron was abnormally low: 60 gamma; total binding capacity 160 gamma. Osmotic fragility was 5.2 to 3.4. Mechanical fragility was normal, but incubation of coagulated blood at 37 C. showed very high fragility. Incubation of heparinized blood showed normal fragility. The acid and Crosby tests were intermittently positive. In March, 1952, a hemorrhagic syndrome with thrombocytopenia was observed. Transfusions with washed red cells were given at regular intervals but the anemia remained at about 3,000,000 RBC per cu. mm. Leukopenia became steadily more severe, around 2000 leukocytes per cu. mm., with 50 per cent neutrophils.

The bone marrow showed a marked erythroblastic reaction (48 per cent) with maturation of the granulocytic series arrested at the myelocyte stage.

Case 612. 13

Acute aleukemic leukemia. This case has been reported elsewhere.

Case 7

Acute aleukemic leukemia. In March 1952, Mlle. R., aged 16, presented bilateral enlargement of the cervical lymph nodes; these disappeared after six sessions of irradiation. In May 1952 anemia appeared and by the end of July 1952, was of severe degree. There was no adenopathy or splenomegaly, no bone pain, and no hemorrhagic phenomena. Blood count: RBC 2,150,000 per cu. mm.; WBC 5800 per cu. mm.; platelets 104,000 per cu. mm. Differential count: neutrophils 8 per cent, eosinophils 0.5 per cent, lymphocytes 75.5 per cent, lymphoblasts 18 per cent. The bone marrow showed invasion by undifferentiated blast cells. Clinical remission occurred after treatment with cortisone and aminopterin. The patient died in June 1953.

Case 82

Hodgkin’s Disease. In 1951, Mme. S., aged 38, presented enlargement of a single axillary lymph node, which on biopsy showed typical lesions of malignant granulomatosis. One year later a second phase began, characterized by dysphagia and pruritus. There were hepatomegaly, splenomegaly, and generalized lymphadenopathy. Blood count: RBC 3,600,000 per cu. mm.; WBC 3000 to 5000 per cu. mm. with 82 per cent neutrophils, 16 per cent monocytes, 2 per cent lymphocytes; platelets 120,000 per cu. mm. Bone marrow: cellularity normal, polymononuclears 18 per cent; other granular cells 42 per cent; lymphocytes 18 per cent; monocytes 5 per cent; erythroblasts 17 per cent. Initial treatment with colchicine brought about temporary remission of fever, but subsequent treatment with triethylene melamine, nitrogen mustard, ACTH, and cortisone gave no results. Autopsy confirmed the diagnosis of Hodgkin’s disease.

Case 9

Lymphosarcoma. Mr. R., aged 41, previously well except for frequent pharyngitis, noted bilateral axillary and supraclavicular adenopathy in 1951. In March 1952, regular tumefactions deformed the face in the region of the cheeks. In May 1952, hemolytic jaundice appeared. The first blood examination, before any treatment, showed RBC 2,720,000 per cu. mm., leukocytes 3600 per cu. mm. with 51 per cent neutrophils. Platelets 132,000 per cu. mm. The bone marrow was hypoplastic and the differential showed polymononuclears 11 per cent, other granular cells 21 per cent, lymphocytes 45 per cent, monocytes 1 per cent, erythroblasts 17 per cent. Lymph node biopsy showed numerous lymphocytes and invasion of the capsule by lymphocytes and lymphoblasts but no Dorothy Reed cells. None of the signs of Brill-Symmers’ disease was present. Irradiation was given, along with transfusions. The general condition, splenomegaly, and adenopathies were not influenced. Treatment with two courses of cortisone 150 mg. daily, lasting thirty-three and fifteen days, gave spectacular but temporary results.
LEUKOAGGLUTININS

Case 10

Lymphosarcoma. Mme. D., aged 56, had been well until the menopause. In 1951, increasing asthenia was noted, with pallor and a slight fever. Blood count: RBC 3,420,000 per cu. mm.; WBC 2200 per cu. mm., with 70 per cent neutrophils, 4 per cent eosinophils, 7 per cent lymphocytes, 19 per cent monocytes. By 1952, bilateral axillary, supraclavicular, and inguinal lymph node enlargement had appeared. The spleen descended to the umbilicus and the right iliac crest. The liver was considerably enlarged. Leukopenia persisted, and at this time the bone marrow was partially invaded by atypical lymphocytes (70 per cent). Later, in spite of multiple transfusions, anemia with leukopenia and thrombopenia increased, reaching 2,600,000 RBC, 2000 leukocytes, and 45,000 platelets per cu. mm. Each transfusion produced a considerable reaction. In July 1953, the patient was splenectomized. The spleen weighed 2000 Gm. and the pathologic examination showed large macrophages and invasion by lymphoblastic cells. After operation, the leukocyte count reached 9000 per cu. mm., with 60 per cent granulocytes.

Cases 11, 12, 13, 14, 16, 1815, and 15, 1713

Chronic idiopathic pancytopenias. These cases have been reported elsewhere.

Case 19

Chronic idiopathic pancytopenia. Mr. B., 52 years old, past medical history negative, was hospitalized in 1952 for aplastic anemia. Blood count: RBC 3,130,000 per cu. mm.; WBC 1000; color index 1.05. Differential: polymorphonuclear cells 6 per cent, monocytes 3 per cent, lymphocytes 91 per cent. The bone marrow was hypoplastic, with only 7.5 per cent of granulocytic elements. The anemia with granulocytopenia had developed over the course of about two years and had been treated by successive transfusions and by cortisone without appreciable amelioration. At present, the patient has a reaction after each transfusion.

REFERENCES

40 I. En Au NAY, LINDSTRÖM, H. J. 25 ANTWEILER, WITEBSKY, E. 38

FnLITTI-WERMER, S., METCINNIKOFF, E.: Sur les cytotoxines. Ansns. miss. Pasteur (1:
AUBEL-LESURE, TINSIENRF, FLECK, 27 ZERMATI, 32 SPAT, ASHBY, 29 DUBERT, J. M., BESSIS, 26

NESCZZ, S. C., Bonn, 30 U.: Experimental observations on chronic agranulocytosis. Quart
EXKRE, - 20 AND ROBINEAUX, R. 21 GUILLOT, _, 15 -

MMEssnEter, I.: Leucopenie chronique par auto-anticorps. Acta

TuLLIS, J. L., TwrBnE, E. A., Wclsnsschr. 27: Insmunso-plsagocytose
14. GoumsnnT, H. 13 J. r2 DAISSET, AN1) DnA1oNn, ANt) VAN LOGHEM,


Bessis, M.: Personal communication.


Leukoagglutinins: V. Leukoagglutinins in Chronic Idiopathic or Symptomatic Pancytopenia and in Paroxysmal Nocturnal Hemoglobinuria

J. DAUSSET, A. NENNA and H. BRECY