Mixed Agglutination of Leukocytes and Erythrocytes in Relation to Studies of Leukocyte Antigens

By Richard F. Bakemeier and Scott N. Swisher

STUDY OF THE antigenic structure of leukocytes and detection of "leuko-agglutinins" have concerned investigators in immunology and hematology for many years. Recently, reports of "leukoagglutinins" in various pathologic conditions have appeared in rapidly increasing numbers, as reviewed by Dausset. Various serologic techniques have been employed, both in the detection of these leukocyte agglutinating factors and in the demonstration of leukocyte antigens. Precipitation methods have been applied to this problem, but the most widely utilized technic has involved the demonstration of direct agglutination of leukocytes. This method has also been employed in conjunction with the absorption of leukocyte agglutinating factors from sera by leukocyte suspensions. Various workers have concluded that human leukocytes possess A and B antigens corresponding to those of the donor's erythrocytes, based upon absorption of anti-A and anti-B by leukocyte suspensions, or the direct agglutination of leukocytes by anti-A and anti-B.

A recent report by Riis describes the application of a direct agglutination procedure to the problem of demonstrating leukocyte antigens. Human leukocytes were found to clump together in anti-A and anti-B sera in accordance with the erythrocyte ABO groups of the leukocyte donors, and the conclusion was drawn that the A and B antigens are present on the leukocytes as they are on the erythrocytes. The anti-A and anti-B sera used by Riis were prepared from blood of group B and group A donors, respectively, after immunization with soluble A and B substances.

This paper describes results of experiments carried out while attempting to apply Riis' methods of leukocyte separation and direct agglutination to the study of the antigenic structure of leukocytes. It has been found that such direct agglutination technics, employing suspensions of leukocytes which also contain erythrocytes, are subject to serious misinterpretation of results. "Agglutination" of human and canine leukocytes which does not correspond to the agglutination reactions of the donor's erythrocytes has been consistently demonstrated with high titer human immune type anti-A sera and with dog sera containing an immune erythrocyte iso-antibody, canine anti-A, respectively.* This clumping, instead, has been seen to be related not to the blood group of the leukocyte donor but to the presence of erythrocytes or erythrocyte ghosts which react with the antiserum present. Such "leukocyte agglutinates," as they appear to be when viewed by ordinary microscopy, have been shown by phase microscopy to be...
914  MIXED AGGLUTINATION OF LEUKOCYTES AND ERYTHROCYTES

mixed clumps of leukocytes and erythrocyte ghosts. The conditions under which this potential source of error may occur in serologic tests for leukocyte antigens and leukocyte agglutinating factors have been investigated.

MATERIALS AND METHODS

Cell Suspensions:

"Leukocyte suspensions" were obtained from freshly drawn venous blood, using siliconized glassware throughout*, with sedimentation in the presence of dextran ("Plavolex" 6%; dextran in 0.9% saline),† by the method of Riis.⁹

A modification of this technic was employed in most experiments to eliminate dextran and/or complement from the suspensions. This involved centrifugation of the suspensions at 80 × G. for ten minutes, after which the clear supernate was replaced with an equal volume of either the cell donor’s fresh serum or the donor’s serum which had been heated at 56 C. for 30 minutes and cooled. The cells were always easily resuspendable. The suspensions thus prepared usually contained approximately equal numbers of leukocytes and erythrocytes (6000 to 10,000 per cu.mm.).

"Erythrocyte suspensions" were obtained from finger punctures; the red cells were washed three times in 0.9% saline, and the buffy coat was removed after each successive centrifugation. No leukocytes were visible upon microscopic examination of these suspensions.

Erythrocyte "ghosts" were prepared by a modification of the method of Ballentine,* using distilled water and flocculation with acetate buffer. There was no clumping of the twice-washed ghosts before use in the experiments.

Sera:

Sera used as “antisera” were collected by sterile venipuncture and stored at -20 C. after separation. Sera used to resuspend leukocytes were obtained concurrently with the blood used for leukocyte suspensions.

Technic of Agglutination Tests:

The technic employed was essentially that described by Riis.⁹ One drop of the leukocyte suspension was mixed in a siliconized 10 × 60 mm. test tube with two drops of the antiserum. In some experiments these tubes were then incubated. In other experiments either one drop of a 0.2% saline suspension of erythrocytes from a donor different from that of the leukocyte suspension, or one drop of saline was also added, as indicated, before incubation. In this manner erythrocytes which were of a group different from that of the leukocyte donor and which reacted with the antiserum present, could be added in concentrations approximating those of the erythrocytes normally present in the leukocyte suspensions. The tubes were always incubated in a 37 C. waterbath for one hour.

The contents of the tubes were then examined on glass slides either by ordinary light microscopy (both 100X and 430X magnification) or by phase contrast microscopy (440X with dry lens objective and 970X with oil immersion lens). This was done by transferring one or two drops of the contents of the tubes to the slide with a siliconized pipet and allowing the drops to flow over the slide. A cover slip was generally placed over the preparation gently, resting on ridges of vaseline on opposite ends of the slide. Lysis of erythrocytes in the suspensions with acetic acid-methylene blue solution, as was done by Riis, was not carried out.

Clumping of leukocytes and erythrocytes was classified as follows:

+++ Virtually complete clumping
++ A majority of cells clumped

* All pipettes, tubes, and syringes coming in contact with leukocytes were siliconized with Dow Corning 200 Fluid.
† Wyeth Laboratories, Philadelphia, Pennsylvania.
Experimental Results

Experiments with Human Leukocytes and Erythrocytes and Human Iso-antibodies

A. ABO System

1. "Leukocyte agglutination" in the presence of antibody-lysed erythrocytes.

The anti-A serum ("K.K.") used in many of the experiments in this study was that of a "dangerous universal donor," described by Ervin, Christian and Young. This was a human group O serum containing immune-type anti-A1 of high agglutination titer (1:5120 vs. human A1 erythrocytes in serum medium) which acted as an hemolysin and fixed complement with human A1 erythrocytes. The relatively low titer of anti-B was removed by absorption with group B erythrocytes. A second anti-A serum, which had an anti-A agglutinin titer of 1:512, was obtained from a group B donor after immunization with purified A

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Antiserum</th>
<th>Leukocyte Suspension</th>
<th>Human Erythrocytes or Saline Added</th>
<th>Agglutination of WBC</th>
<th>Free WBC</th>
<th>RBC</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Human Anti- A1 &quot;K.K.&quot;</td>
<td>Fresh</td>
<td>Saline</td>
<td>+++++</td>
<td>±</td>
<td>+++</td>
<td>Tightly clumped WBC with few adherent ghosts. No intact RBC</td>
</tr>
<tr>
<td>3</td>
<td>Human Anti- A1 &quot;K.K.&quot;</td>
<td>Fresh</td>
<td>A1 RBC</td>
<td>-</td>
<td>+</td>
<td>++++</td>
<td>Appears as Tube 1, except more ghosts adherent to WBC clumps.</td>
</tr>
<tr>
<td>5</td>
<td>Human Anti- A1 &quot;K.K.&quot;</td>
<td>Fresh</td>
<td>A1 ghosts</td>
<td>-</td>
<td>++++</td>
<td>±</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>Human Anti- A1 &quot;K.K.&quot;</td>
<td>Fresh</td>
<td>Saline</td>
<td>-</td>
<td>-</td>
<td>++++</td>
<td>No agglutination.</td>
</tr>
<tr>
<td>9</td>
<td>Leukocyte Donor A (Group A)</td>
<td>Fresh</td>
<td>Saline</td>
<td>-</td>
<td>-</td>
<td>++++</td>
<td>No agglutination.</td>
</tr>
<tr>
<td>10</td>
<td>Leukocyte Donor A (Group A)</td>
<td>Heat-inact.</td>
<td>Saline</td>
<td>-</td>
<td>-</td>
<td>++++</td>
<td>No agglutination.</td>
</tr>
</tbody>
</table>

* RBC—erythrocyte
† WBC—leukocyte
MIXED AGGLUTINATION OF LEUKOCYTES AND ERYTHROCYTES

substance. This serum was similar to serum “K.K.” but agglutination was less pronounced. The anti-B serum “D.M.” was the serum of a group A donor who had been immunized with purified B substance, and demonstrated an anti-B agglutinin titer of 1:512.

Agglutination tests as described under “Materials and Methods” were performed, using leukocyte suspensions from donors of groups A, B, AB, and O, both in fresh autogenous serum and in heat-inactivated autogenous serum; erythrocyte suspensions of groups A and B; and the sera described above. Tests were read both by ordinary light microscopy and by phase contrast microscopy. Representative results are summarized in table 1.

These results show that the leukocytes from donors of either group A (four tested) or group O (ten tested) agglutinated in the presence of immune anti-A and fresh serum, if group A erythrocytes were present. Although phase microscopy revealed that many of these “leukocyte clumps” contained erythrocyte ghosts, ordinary light microscopy did not reveal the mixed nature of the agglutinates. In a heat-inactivated serum system, either method of microscopy readily revealed mixed agglutinates consisting of leukocytes from either group A or group O donors, as the case may have been, and group A erythrocytes.

Figures 1–4 are photomicrographs of representative phase microscopic fields from these experiments. Figure 1 shows a field from Tube 7, table 1, with isolated

![Fig. 1.—Photomicrograph of a representative field from Tube 7, table 3, showing isolated leukocytes and erythrocytes from a human group O donor, in strong, immune-type human anti-A with fresh serum present. Phase contrast, (43 X obj.; 10 X eyepiece)
leukocytes and erythrocytes from a group O donor, in anti-A serum. Figure 2 represents Tube 3, table 1, showing a “leukocyte clump” consisting of leukocytes from a group O donor, in the presence of A1 erythrocytes, anti-A serum, and fresh serum. Unfortunately, the photograph does not define the adherent ghosts satisfactorily. Ghosts can be seen best by focusing up and down on these mixed clumps. Figure 3 represents Tube 4, table 1, and shows a mixed clump of leukocytes and intact erythrocytes. The mixture in this tube is the same as that in figure 2, except that inactivated serum has been used instead of fresh serum, thereby preventing antibody lysis of the A1 erythrocytes.

The human B erythrocyte-anti-B system demonstrated much less clumping of leukocytes from group B or group O donors, in the presence of antibody-lysed group B erythrocytes, than was seen in comparable experiments employing the human A erythrocyte-anti-A system. This may have been due to the comparatively low antibody titer in the anti-B serum. However, marked mixed clumping of group B erythrocytes with leukocytes from either group B or group O donors was observed in the presence of the immune anti-B serum in tubes not containing fresh serum.
Results obtained with the leukocytes of one group AB donor were similar to those obtained using group A or group B cells and the corresponding antiserum. Commercial anti-A and anti-B sera* did not appear to produce appreciable mixed clumping of leukocytes with agglutinated erythrocytes or pure leukocyte clumping; a few leukocytes were adherent to the peripheries of some erythrocyte clumps.

2. "Leukocyte agglutination" in the presence of osmotically-lysed erythrocytes. Human group A₁ erythrocyte ghosts were prepared as described under "Materials and Methods" and were added to antiserum-leukocyte suspension (group O) mixtures in place of intact A₁ erythrocytes as used in (1). This was done to simulate the conditions present in certain leukoagglutination technics employing osmotic or chemical lysis of erythrocytes.

The results in table 1, rows 5 and 6, show that the presence of A₁ erythrocytes lysed by an osmotic technic produced a picture of marked aggregation of leukocytes from a group O donor in the presence of human immune anti-A₁ serum and either fresh or heat-inactivated autogenous serum. Under phase microscopy these aggregates were easily seen to be composed of both erythrocyte ghosts and leukocytes.

* Scientific Products Division, American Hospital Supply Corp., Evanston, Ill.
3. Demonstration of mixed leukocyte-erythrocyte clumping with “normal” human sera. The phenomenon of mixed clumping of leukocytes and erythrocytes, as described in (1) in inactivated serum systems containing immune anti-A or anti-B, was also noted in examining certain of the sera in a series of healthy adult male group O donors and one group B donor, none of whom had ever received a blood transfusion or injections of purified A substance. “Leukoaagglutinating” properties with leukocytes from a group A1 donor, and anti-A1 erythrocyte agglutinin and complement-fixing antibody titers, respectively, were determined independently by the author and a qualified technician. The results are given in table 2.

All sera caused agglutination of the A1 erythrocytes. The mixed clumping of erythrocytes and leukocytes seen with sera “J.E.” and “C.B.” was appreciably diminished when inactivated autogenous serum was used in place of fresh autogenous serum in the leukocyte suspension, while that small degree of mixed clumping seen with sera “J.B.” and “F.S.” was not seen at all in the inactivated serum systems.

Figure 4 is representative of results presented in table 2 and shows a mixed
MIXED AGGLUTINATION OF LEUKOCYTES AND ERYTHROCYTES

Table 2

<table>
<thead>
<tr>
<th>Human Serum (All except “F.B.” Group O)</th>
<th>WBC Agglutination (As Donor)</th>
<th>Mixed As RBC-WBC Agglutination</th>
<th>Anti-A Agglutinin Titer</th>
<th>Anti-A Comp.-Fix’n. Titer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>“K.K.”</td>
<td>+ + +</td>
<td>+ + +</td>
<td>5120</td>
<td>128</td>
</tr>
<tr>
<td>“J.E.”</td>
<td>±</td>
<td>+ + +</td>
<td>128</td>
<td>64</td>
</tr>
<tr>
<td>“C.B.”</td>
<td>±</td>
<td>+ + +</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>“J.B.”</td>
<td>−</td>
<td>+ †</td>
<td>64</td>
<td>4</td>
</tr>
<tr>
<td>“F.S.”</td>
<td>−</td>
<td>± †</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>“T.G.”</td>
<td>−</td>
<td>−</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>“Lit.”</td>
<td>−</td>
<td>−</td>
<td>32</td>
<td>Not tested</td>
</tr>
<tr>
<td>“C.R.”</td>
<td>−</td>
<td>−</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>“F.B.” (Group B)</td>
<td>−</td>
<td>−</td>
<td>16</td>
<td>0</td>
</tr>
</tbody>
</table>

* Reciprocal of highest dilution of serum causing fixation of guinea pig complement in the presence of human A1 erythrocytes.
† WBC not intermixed with RBC but were adherent to RBC agglutinates in large numbers.

clump of leukocytes from a group O donor and A1 erythrocytes, in the presence of “normal” group O serum (from the leukocyte donor) and fresh serum.

Thus, it was seen that the appearance of “mixed agglutination” of erythrocytes and leukocytes of a group A donor can occur in the sera of certain supposedly non-immunized group O individuals. This phenomenon appears to be associated with (1) relatively high anti-A agglutinin titer and/or (2) certain “immune” characteristics (i.e., the ability to fix guinea pig complement in the presence of group A1 erythrocytes). Fresh serum appears to enhance this “mixed agglutination.”

B. Other Human Blood Group Systems

Experiments similar to those described involving the human ABO system were conducted with erythrocytes and leukocytes from donors representing different groups in the Rh system and in the Lewis system.

Sera from three cases of isoimmunization of pregnancy, one containing anti-C (anti-Rh’) and two containing anti-D (anti-Rh0) were studied for ability to produce “leukoagglutination” and mixed erythrocyte-leukocyte agglutination. No significant agglutination of leukocytes or mixed agglutination of erythrocytes and leukocytes obtained from persons of either group O Rh-positive (CDe) or group O Rh-negative (Cde) was seen, even in the presence of antibody-agglutinated erythrocytes in systems containing either fresh or inactivated serum.

An anti-Le(a+) serum was obtained from a patient following a transfusion reaction involving this system. It demonstrated an agglutinin titer for group O Le(a+) erythrocytes of 1:16 in serum medium, and fixed guinea pig complement in the presence of these erythrocytes. Mixed clumping of group O Le(a+) erythrocytes with leukocytes from group O donors occurred in the presence of fresh serum and anti-Le(a+), regardless of whether the leukocytes were from Le(a+) or Le(a−) donors. This mixed clumping was not seen in heat-inactivated serum systems. No homogeneous-appearing clumps of leukocytes were observed in these experiments. The Le(a+) erythrocytes were only agglutinated, not lysed, precluding the clumping of leukocytes around erythrocyte ghosts.
EXPERIMENTS WITH CANINE LEUKOCYTES AND ERYTHROCYTES AND CANINE ISO-ANTIBODIES

A. Canine A Erythrocyte-Anti-A System

Canine anti-A as described by Christian, Ervin, and Young, is a thermostable, complement-fixing, hemolytic antibody which sensitizes erythrocytes for the anti-globulin test, thereby resembling certain immune human iso-antibodies.

Antisera were prepared by immunizing dogs possessing A-negative erythrocytes with canine group A1 erythrocytes. Leukocyte and erythrocyte separation and agglutination tests were carried out as described under “Materials and Methods.”

It was observed that leukocytes from dogs with group A1 erythrocytes were markedly clumped in canine anti-A1 serum. However, a very similar picture, indistinguishable from the preceding when viewed under an ordinary light microscope, was obtained with leukocytes from a dog with A-negative erythrocytes when A+ red cells were also added to the system. Under phase microscopy the “leukocyte agglutinates” in both experiments were seen to be mixtures of leukocytes and erythrocyte ghosts, comparable to the mixtures of leukocytes and intact erythrocytes seen with the same reagents in the absence of fresh serum. These results were entirely comparable to those obtained with human anti-A and cell suspensions from A-positive and A-negative donors.

B. Canine D Erythrocyte-Anti-D System

Tests corresponding to those involving the canine A-anti-A system were carried out with leukocyte and erythrocyte suspensions from canine group D donors and anti-D serum. Canine anti-D is a non-complement-fixing, non-hemolytic antibody which does not sensitize erythrocytes for the anti-globulin test, thereby resembling naturally-occurring human iso-antibodies. No clumping or mixed agglutination of leukocytes from dogs with either D-positive or D-negative erythrocytes occurred in anti-D serum, even in the presence of agglutinated D-positive erythrocytes.

Discussion

The results presented in this paper demonstrate potential sources of error in the interpretation of leukoagglutination, which, while already recognized in the literature, are nevertheless inherent in techniques currently being employed in the analysis of leukocyte antigens and in the detection of “leukoagglutinins.”

Direct agglutination procedures employing leukocytes are made technically more difficult to perform than hemagglutination and even, perhaps, than platelet agglutination procedures by the physiologic tendency of leukocytes to be attracted to foreign particles, to contaminating bacteria (Goudsmit and van Loghem), and to cells damaged by antibody sensitization or other, non-specific trauma. This source of difficulty is apparent in techniques employing “leukocyte suspensions” which actually are mixed suspensions of leukocytes and erythrocytes. In such suspensions erythrophagocytosis or the antecedent process, adherence of leukocytes to damaged erythrocytes, is a phenomenon which must be considered in interpreting any form of leukoagglutination. This is especially important when sera, which are being examined for antibodies directed
against leukocyte antigens, also contain, or are not known to be free from, antibodies against the erythrocytes inevitably present in the leukocyte suspensions. Prior absorption of the erythrocyte antibodies of the “antiserum” with pure erythrocyte suspensions from the leukocyte donor can be employed in certain instances if erythrocyte antibodies are present, providing any erythrocytes that are sensitized and lysed during the absorption procedure are removed before testing the serum with leukocytes. However, when the problem under investigation is the detection of antigens on the leukocytes which are the same as those known to be on the erythrocytes, such as ABO and Rh system antigens, this precautionary procedure cannot be utilized, except as a means of demonstrating specific absorption of antibody by leukocytes.

It has been shown that ordinary light microscopy is not generally capable of distinguishing, as such, clumps of leukocytes which are agglomerated around antibody-sensitized erythrocyte ghosts. It may even be difficult to detect such mixed clumping with phase microscopy when the leukocytes are tightly clumped together. Therefore, when conditions exist which permit such mixed clumping of leukocytes with lysed or intact erythrocytes, any observed leukoagglutination should be interpreted with extreme caution.

Conditions under which experiments involving leukoagglutination are subject to this source of difficulty can be determined from the results presented in this paper and from studies of erythrophagocytosis carried out by other workers. Two of these conditions appear to be:

1. The presence of a complement-fixing, potentially hemolytic red cell antibody in the serum to be tested for the presence of leukocyte agglutinins.
2. The presence of thermolabile components of fresh serum in the mixture of reagents used in the leukoagglutinating tests.

Mixed clumping of leukocytes and erythrocytes was seen in the presence of complement-fixing human anti-A, canine anti-A, and in an anti-Le(a) serum which contained complement-fixing antibody. However, no mixed clumping was seen in human group O sera not containing complement-fixing anti-A, or in three Rh anti-sera, which did not demonstrate complement fixation. These results, when compared with the observations of Bonnin and Schwartz\(^{15}\) on in vitro erythrophagocytosis, suggest that this mixed agglutination of leukocytes and erythrocytes is related to the phenomenon of erythrophagocytosis. Bonnin and Schwartz reported that “…erythrophagocytosis could only be elicited with antibodies that were actually or potentially hemolytic.” They found that Rh antibodies did not cause erythrophagocytosis. Harkink, Doorman, and van Loghem\(^{15}\) obtained similar results in their studies of erythrophagocytosis involving ABO, Rh, Kell, Duffy, MN, and P systems.

Butler reported a “significant” erythrophagocytic index in 22 of 24 incompatible combinations of plasma samples and leukocyte suspensions which showed mixed clumping of erythrocytes and leukocytes. An hemolysin was demonstrated in only one of seven group O sera from donors whose plasma produced such mixed clumping; however, complement fixation tests were not reported.

The presence of thermolabile components of fresh serum permitted lysis of erythrocytes in the experiments involving human and canine anti-A, with the
formation of mixed leukocyte-erythrocyte ghost clumps appearing as "pure" leukocyte agglutinates when viewed with ordinary microscopy. Heat inactivation of all sera present prevented this hemolysis and the mixed nature of the clumps became apparent. In the experiments involving the anti-Le(a) serum and the "normal" human O sera containing relatively low titers of complement-fixing anti-A, heat inactivation of serum eliminated mixed erythrocyte-leukocyte clumping seen in the presence of fresh serum.

Several leukoagglutination technics currently employed in a number of laboratories include one or both of these elements which may contribute to immunologically non-specific clumping of leukocytes. Experiments employing strong immune human anti-A and anti-B sera, human "leukocyte suspensions" containing erythrocytes as well, and fresh, defibrinated plasma, in essentially the same manner as in the study presented in this paper, have recently been described in the literature. Clumping of erythrocytes from donors of different ABO groups was reported to occur in accordance with the agglutination reactions of the corresponding erythrocytes. The conclusion drawn from those experiments was that this clumping demonstrated the presence of A and B antigens on the leukocytes. Failure of leukocytes to participate in the agglutination of human D-positive (Rh+) erythrocytes in anti-D serum was interpreted as evidence in support of the concept that leukocytes lack the D antigen. These data appear to deserve re-evaluation, in the light of the data presented here. The leukocyte agglutinates observed in Rii's experiments consisted mainly of the potentially phagocytic granulocytes, while most of the non-agglutinated leukocytes were lymphocytes. This observation strengthens the possibility that erythrophagocytosis was involved and could not be detected by the ordinary light microscopy employed because of lysis of erythrocytes with acetic acid-methylene blue solution. The failure to produce leukoagglutination with human anti-D may represent the inability of this antibody to induce immunologically nonspecific mixed agglutination in vitro, or the absence of sufficient antibody to do so, and not the absence of D antigen sites on the leukocytes.

Conversely, mixed agglutination of erythrocytes and leukocytes, in the presence of erythrocyte antibodies, apparently should not be interpreted as definitive evidence that antigens common to the erythrocytes and leukocytes are being "linked" by the corresponding antibodies, especially when fresh serum and or immune-type, potentially hemolytic antibody are employed in the test system. Rigorous controls must be included in experiments involving any type of mixed cell agglutination. One of the authors (S.N.S.) has demonstrated nonspecific clumping of platelets around erythrocytes exposed to the action of complement-fixing erythrocyte antibodies, both in vivo and in vitro, as an example of this phenomenon involving another blood element.18

Conclusions

It appears that investigations of leukocyte antigenic structure, or of "leukoagglutinins"17 in various pathologic conditions, by serologic methods which may result in "mixed agglutination" of leukocytes and either intact or lysed erythrocytes, are subject to serious misinterpretation if such agglutination reactions
are considered to be immunologically specific, especially in the presence of thermolabile serum components and/or potentially hemolytic antibody. More satisfactory application of direct agglutination procedures in leukocyte serology would be greatly facilitated by the development of better methods of isolating leukocytes from other cellular blood elements.

Summary

Serologic experiments are described in which was studied the agglutination of leukocytes from donors representing various ABO, Rh, and Lewis erythrocyte groups and certain canine blood groups, in the presence of corresponding antisera.

Appropriate mixing of antisera with leukocytes and erythrocytes from donors of different groups was seen to produce clumping of leukocytes which did not conform to the reactions of the erythrocytes from the leukocyte donor. When viewed under phase microscopy, certain of these leukocyte clumps, which appeared homogeneous with ordinary light microscopy were found to be clumps of leukocytes mixed with ghosts of erythrocytes reacting with the antibody present.

Factors contributing to this apparently immunologically non-specific clumping were the presence of complement-fixing, potentially hemolytic antibody and thermolabile components of serum. A possible relationship with erythrophagocytosis is suggested.

These observations indicate that certain results of this and other “leuko-agglutination” techniques, which have been interpreted as demonstrating the presence of A and B antigens on human leukocytes, deserve re-evaluation and emphasize the importance of developing methods of preparing homogeneous leukocyte suspensions.

Summario in Interlingua

Es describite experimentos serologic serviente le studio del agglutinacion de leucocytos ab donatores representante varie gruppos erythrocytic ABO, Rh, e Lewis e plure gruppos sanguinee canin in le presentia del correspondente antiseros.

Esseva notate que le appropriate mixtion de antiseros con leucocytos e erythrocytos ab donatores de differente gruppos produceva agglutination de leucocytos non de acordo con le reactiones del erythrocytos ab le donator del leucocytes. Sub le microscopio phasic, certe de iste coagulos leucocytic—que se mostrava homogenee in microscopia a lumine ordinari—se revelava como mixturas de leucocytos con spectros de erythrocytos in reaction con le anticorpore presente.

Factores contributori in iste coagulation (que pareva esser immunologicamente non-specific) eseva le presentia de anticorpore potentialmente hemolytic e characterisate per su fixation de complemento e le presentia de componentes thermolabile del sero. Es suggerite le possibilitate de un relation con erythrophagocytosis.

Iste observationes indica que certe resultatos de iste e altere tecnicas “leuco-agglutinatori”—le quales ha essite interpretate como demonstration del presentia de antigenos A e B super leucocytos human—merita esser re-examine. Le observationes etiam sublinea le importantia del disveloppamento de methodos pro le preparation de homogenee suspensiones de leucocytos.
REFERENCES

Mixed Agglutination of Leukocytes and Erythrocytes in Relation to Studies of Leukocyte Antigens

RICHARD F. BAKEMEIER and SCOTT N. SWISHER