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THE ROLE OF LEUKOCYTE ANTIBODIES
IN LEUKOPENIC STATES

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of
Master of Science
University of Saskatchewan

by
Avril Virginia Russell

Saskatoon, Saskatchewan
March 31, 1961

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CHAPTER I

INTRODUCTION

Paul Ehrlich was the first to put forth the concept of the "horror autotoxicus", which is the inability of the body to form antibodies against its own components. A convincing demonstration of this concept is seen in the homograft reaction. Evidence has been accumulating for many years that under certain circumstances the body can respond immunologically to some of its own antigens. This topic is very controversial and none of the theories and proofs offered seem entirely adequate. Immunological studies have, however, established the phenomena of antibody production against foreign substances such as proteins, erythrocytes and bacteria. Yet, comparatively little is known about humoral immune systems involving nucleated mammalian cells, for example, the leukocyte, the epithelial cell and the fibroblast, to name only a few.

The leukocyte is rather well adapted to investigation, as it is somewhat easier to obtain in pure form than most other body cells, and knowledge of its physiologic and biochemical properties is reasonably broad. Aberrations under the impact of an immune reaction are therefore amenable to investigation.

The frequent occurrence of idiopathic leukopenias focused attention on the possibility of leukocyte antibodies. Many leukopenic states have no satisfactory scientific explanation and
are potentially serious to the patient. An explanation of the mechanism of these leukopenic states may lead to proper diagnosis and therapy.

It is also feasible that the understanding of leukocyte antigens might be of importance for the late results of bone marrow transplantation and also may have some bearing on the problems of tissue transplantation.

In 1952 Dausset and Nenna described a homologous anti-leukocyte antibody in the serum of a patient with agranulocytosis. In the same year, Moeschlin and Wagner reported the association of leuko-agglutination with pyramidon sensitization in the human. Since these two reports, a large literature has accumulated and was recently fully reviewed by Walford, who listed over five hundred reports. Most of these are chiefly concerned with establishing the presence of such antibodies, the mechanisms by which they cause pathological effects being still controversial. Leukocyte antibodies have been implicated in the production of certain transfusion reactions, the L.E. phenomena, some instances of skin and tumour homografting, and in certain neutropenic states.

Most frequently, iso-immunization to leukocytes has been described as the sequel of multiple transfusions. In this study, patients with leukopenic states and histories of multiple transfusions were investigated.

Payne and Rolfs have demonstrated the occurrence of leukocyte antibodies as a result of sensitization by foetal leukocytes. These authors were unable to attribute any pathological effects of
such antibodies on the bone marrow or white cell count of the infants. Other authors 10,11,12,13 have also postulated that neutropenic states in the newborn could be the result of the actions of maternal iso-immune antibodies on the foetus, but without direct evidence.

One of the purposes of this study was to aid in clarifying the mechanism of neonatal neutropenia. A family with multiple cases of neonatal neutropenia and with a further pregnancy presented an excellent opportunity for investigation.

If Koch's Postulates are applied to the phenomena of leukocyte antibodies in order to establish the causal relationship of the antibodies to the leukopenic states, it is necessary that the antibody be found in all cases of the disease in question, that the antibody be produced outside the body of the host, and that the antibody so produced should reproduce the disease in other susceptible animals. An attempt was made in this study to fulfil these criteria.

The ability of the polymorphonuclear leukocyte to phagocytose and digest invading bacteria is a characteristic phenomena and is important in combatting infections. Although leukocyte antibodies are usually demonstrated by in vitro agglutination of the leukocytes, it does not follow that these agglutinated leukocytes have lost their phagocytic function. The necessity of demonstrating a loss of phagocytic function is paramount to establishing the destructive effect of leukocyte antibodies. Tullis' method 14 of counting the number of leukocytes with pseudopodia before and after
incubation with the immune serum approaches this problem. An improvement of this method was made in this study by including bacteria in the system and then counting the number of engulfed bacteria before and after incubation of the leukocytes with the immune serum.

The leuko-agglutinin test was the main technique used in this study for demonstrating leukocyte antigen-antibody reactions, as it is the most widely used test by other investigators. By utilizing this common test the findings of this study could be correlated with those of other investigators. Nevertheless, other methods were sometimes applied in conjunction with the leuko-agglutinin test to illustrate pertinent points.
Neutropenia in the newborn has been sporadically reported in the past,\textsuperscript{10,11} but the underlying pathology has never been disclosed. Payne and Rolfs\textsuperscript{9} have demonstrated the occurrence of leukocyte antibodies as a result of sensitization by foetal leukocytes, but these authors were unable to attribute any pathological effects of such antibodies on the bone marrow or white cell count of the infants. In 1954 Ballowitz and Ballowitz\textsuperscript{12} reported a case of erythroblastosis foetalis (anti-C, anti-D) in which the child recovered following exchange transfusion. During the subsequent three months of its life, the infant had a marked leukopenia. The maternal serum inhibited marrow cultures from subjects with C and D type blood. Other authors\textsuperscript{10,13} also postulated that neutropenic states in the newborn could be the result of the actions of maternal iso-immune antibodies on the foetus, but without direct evidence.

A pregnant woman with a past history of neonatal neutropenia in her children presented an excellent opportunity for investigation. The object was to discover if leukocyte antibodies were present in the mother, present in the child, and if so what effect they had on the child's leukocytes and on the leukocytes of the other siblings.
I. CASE REPORT

These data were obtained following the most recent pregnancy of a thirty-two year old woman of Anglo-Saxon origin. Her first pregnancy was uneventful. The second terminated in a stillbirth of approximately six months gestation, and shortly after this the mother received a transfusion of fresh whole blood donated by her husband. The third pregnancy was uneventful. The fourth child was born at full term, but contracted a neonatal infection with marked neutropenia and died of septicaemia after fifteen days of life. The fifth child was normal. The sixth child had neonatal neutropenia and a severe infection, but recovered after treatment with antibiotics and adrenal cortical steroids. The neutrophil count of this child rose to normal levels during treatment for the infection.

In view of the past events, blood was obtained from the pregnant mother before and after delivery.

The baby was born at full term, weighed eight pounds and was normal on physical examination. The white blood cell count at birth was 17,000 per cu.mm. Four hours after birth the white blood cell count had dropped to 8,000 per cu.mm. with only 27 per cent of these cells in the granulocytic series. During the neutropenic period the white blood cell count fluctuated between 5,600 per cu.mm. and 13,200 per cu.mm. with 1 to 48 per cent granulocytic cells. Up to six months after birth the diminished number of neutrophils was apparently compensated by an increased number of monocytes. The
latter diminished as the neutrophils slowly increased. Lymphocytes were always within normal limits.

Tibial bone marrow aspirations were performed on the eighth and twenty-eighth days of life. The first showed a marked reduction of all leukocytic elements. The second showed hyperplasia most prominently in the myeloid and monocytic series. In both aspirations, marked dilution with peripheral blood made the differential count meaningless.

Seven days after birth a small bleb was noticed on the child's face and white patches in its mouth. Candida albicans was cultured from both areas. The child was placed on strict isolation and treated with Mycostatin and the lesions cleared. Apart from a brief period of fever and diarrhoea, which responded to Neomycin, the child's progress was satisfactory.

II. MATERIALS AND METHODS

Preparation of leukocyte suspensions. Suspensions of leukocytes were obtained from the mother, the father, and all surviving children, except the baby, from whom it was impossible to obtain adequate numbers of cells. The blood was taken by venipuncture using a two syringe technique.

All the glassware used in this procedure was siliconed with Drifilm 88, using a vaporizing technique. Needles were treated with "Arquad".

The blood samples were defibrinated and 8 ml. of the defibrinated blood were added to 2.5 ml. Dextran 250 (viscosity number
dl/g 0.42) in a 4 per cent w/v solution with 3 per cent dextrose in 8.5 M sodium chloride. This was thoroughly mixed and transferred to a clean test tube placed at a forty-five degree angle and allowed to sediment for forty-five minutes. A 2 ml. quantity of the supernatant leukocyte rich plasma was removed and mixed with 10 ml. isotonic saline in a 150 ml. Erlenmeyer flask. This was allowed to sediment for forty-five minutes, after which 10.5 ml. of the supernatant were removed with a pipette and discarded. The flask was swirled and the suspension examined microscopically. The concentrated cells were not clumped and the granulocytes showed amoeboid activity. The cell suspensions were used immediately.

**Preparation of sera.** Samples of maternal blood were taken twenty-three days and twelve days prior to delivery, and also thirty-five and ninety-three days after delivery. Cord blood was also obtained. Samples of blood were taken from the surviving children and the father. All blood samples were allowed to clot and centrifuged at three thousand revolutions per minute for thirty minutes and the sera removed. All sera were heated for thirty minutes at fifty-six degrees centigrade, since it has been shown that certain sera are inactive unless so heated. The sera were either used immediately or frozen.

**Leuko-agglutinin test.** Sera and leukocyte suspensions prepared as above were used in this test. The sera were set up undiluted and in doubling dilutions in saline from 1:2 to 1:512. Small siliconed test tubes were used. 0.05 ml. of leukocyte suspension was added to 0.1 ml. of each serum dilution. The tubes
were incubated in a thirty-seven degree centigrade water bath for one hour. Each tube was then vigorously tapped twenty-five times against a hard surface. This step served to break up nonspecific clumping without influencing true agglutination. One drop of this suspension was coverslipped on a clean glass slide and examined using phase microscopy. The remainder of the suspension was poured on a glass slide, allowed to dry, fixed by methyl alcohol, stained in the usual manner with Wright's stain and then examined microscopically for evidence of agglutination. The strength of a positive reaction was classified from + to +++, + representing three to five agglutinates per low power field, +++ nearly maximal clumping with very few leukocytes remaining isolated between the agglutinates. (+) was used for very weak agglutination, which, however, differed distinctly from the controls. Negative controls were always run with each serum.

The leuko-agglutinin test was set up using the maternal sera and leukocyte suspensions from the surviving children, the father, the mother, and six unrelated donors. Negative controls consisted of sera and leukocyte suspensions from the same donor.

Test for leukolysins. Tullis' method was used. The leukocytes were suspended in modified Hank's buffer to a concentration of 20,000 to 30,000 per cu.mm. Serum from a healthy Type O, Rh negative donor was filtered through no. 5 Whatman pads, stored in the frozen state, and used as a source of complement. Before use, it was warmed to room temperature and diluted 1:32 with saline.
The leukocyte suspension was drawn to the 0.5 mark of a standard white-cell-diluting pipette. Diluted complement was then drawn to the 1.0 mark, which was followed by drawing serum to the 1.1 mark. The pipette was shaken and a drop was placed on a counting chamber and the number of leukocytes counted. The pipette was sealed and placed in a thirty-seven degree centigrade waterbath and repeat counts were obtained at thirty and sixty minutes. Visible clumps were not counted. A second pipette was drawn up in the same manner. Only zero and sixty minute counts were done from this pipette. When the zero-minute count was plated, another drop was taken from the pipette and used for observation of amoeboid motility under phase microscopy. This was repeated at sixty minutes, from the same pipette. One hundred leukocytes were counted for the amoeboid count. Three categories were noted: (1) amoeboid movement, (2) brownian movement, (3) dead cells. The amoeboid forms showed variation in size and shape of the cells. The percentage decrease in both the total white cell count and the amoeboid motility was noted and used in the calculation of the lysis index. The lysis index is the sixty minute percentage decrease count times the sixty minute percentage decrease in amoeboid motility. A lysis index of zero to twenty-five represents a normal serum.

In this experiment the maternal serum taken twelve days prior to delivery was set up against an unrelated donor's leukocytes. A control system was set up substituting serum from the leukocyte donor for the maternal serum.
Absorption and elution tests. Leukocytes were obtained by Dextran sedimentation of the blood. The leukocyte rich supernatant was centrifuged at two thousand revolutions per minute for ten minutes. The supernatant was discarded and the button of cells washed three times in isotonic saline. One-half ml. of the packed leukocytes was mixed with 0.5 ml. of the immune test serum and left at thirty-seven degrees centigrade for three hours, with occasional mixing. A separate sample of the immune test serum was also incubated at thirty-seven degrees centigrade for three hours. The mixture of serum and cells was then centrifuged at three thousand revolutions per minute for ten minutes and the absorbed serum pipetted off. This absorbed serum and the sample of the same serum prior to absorption, which was incubated separately, were used in the leuko-agglutinin test. A marked fall in the titre of the absorbed serum was taken to indicate absorption of the leukocyte antibodies by the leukocytes.

For the elution studies, the leukocytes used for absorption were washed three times in ten ml. of saline, packed, the remaining saline sucked off with filter paper, and an equal volume of saline added. After thoroughly mixing, this mixture was placed in a waterbath of fifty-six degrees centigrade for thirty minutes and then centrifuged immediately in centrifuge cups previously heated to fifty-six degrees centigrade. The supernatant was quickly removed and freed of possible remaining cells by a second centrifugation. This supernatant was utilized in the leuko-agglutinin test, which if positive was taken to indicate elution of the
leukocyte antibodies from the exposed leukocytes.

The maternal serum and leukocytes from an unrelated donor were investigated for absorption and elution properties. The donor's leukocytes had previously been shown to give positive results in the leuko-agglutinin test with the maternal serum.

III. RESULTS

**Leuko-agglutinin test.** Positive results were obtained with the maternal sera using the father's leukocytes, leukocytes from the previously affected child (sixth born), and with leukocytes from three unrelated donors. Negative results were obtained with the maternal sera, when the mother's leukocytes, leukocytes of the three unaffected children, and leukocytes of three unrelated donors were used. Titres for each sample of maternal serum did not differ by more than one dilution in the five positive leukocyte suspensions. The maternal sera taken twenty-three days and twelve days prior to delivery gave positive results up to titres of 1:64 and 1:512 respectively. The maternal sera taken thirty-five days and ninety-three days after delivery gave positive results up to titres of 1:32 and 1:4 respectively. Thus, a rise in leukocyte antibodies reaching a peak at delivery and then declining, was demonstrated for the maternal sera. The cord blood was positive for leukocyte antibodies up to a titre of 1:16.

**Test for leukolysins.** Leukolysins were demonstrated in the maternal serum. In this test, a fall in the leukocyte count from 20,300 per cu.mm. to 7,150 per cu.mm. occurred after one hour's
incubation of maternal serum and donor leukocytes. The amoeboid motility of the leukocytes decreased twenty-five per cent after incubation of one hour with maternal serum. The lysis index (per cent decrease in count x per cent decrease in motile forms) was thus sixteen hundred, which by Tullis' standards is highly abnormal. \textsuperscript{14} The lysis index of the normal control serum was four.

**Absorption and elution tests.** The absorbed maternal serum was positive for agglutination up to a titre of 1:4, whereas the unabsorbed maternal serum was positive to a titre of 1:512.

IV. SUMMARY

The data obtained in this study indicate sensitization of the mother by her husband's leukocytes with the formation of anti-leukocyte antibodies. Succeeding pregnancies with foetuses bearing the appropriate antigens resulted in further stimulation of maternal antibody. Neonatal neutropenia and infection, causing the death of one of the affected infants, was probably the result of the transplacental transfer of the antileukocyte antibody. In the last pregnancy, rising leukocyte antibody titres were present, reaching a peak just before delivery and declining thereafter. Passive transfer to this infant was demonstrated by the presence of antibodies in the cord blood.

The effect of the antibodies was marked, prolonged, and fairly specific. The neutrophil series was exclusively affected, resulting in neutropenia. The marked rise in monocytes was probably a compensatory mechanism. \textsuperscript{15} These cells declined in
numbers as the neutrophils increased. Lymphocytes appeared unaffected.

One point must be brought out with regard to Tullis' leukolysin test. This point is that the decrease in intact leukocytes in the first pipette might have been due to either agglutination, lysis, or both. No apparent distinction was made between these two manifestations of antibody activity. Also the necessity for complement in the test system is not characteristic of an agglutinin. The leukocyte concentration in the final suspension used was much less than that used in the leuko-agglutinin test. Agglutination would seem much less likely to occur, or at least more difficult to observe, at this low concentration, than with the leuko-agglutinin test. Walford concludes that Tullis' method demonstrates leukolysins rather than leuko-agglutinins.

The erythrocyte types of husband and wife are very similar, particularly with regard to systems which are highly antigenic.

Father:  O MS Ms P+ CDe/CD e kk Fy(a-)

Mother:  O Ns Ns P+ CDe/cDE kk Fy(a+)

The transfusion of the husband's erythrocytes to the wife would have left the wife's antibody forming mechanisms free to form antibodies against weaker antigens such as the husband's leukocytes.

Payne and Rolfs found leuko-agglutinins in the sera of twenty-five of one hundred and forty-five pregnant multiparous women. Of these twenty-five, eighteen had never been transfused. No consistent effect on the newborn of maternal leukocyte iso-agglutinins was noted, and transitory neonatal leukopenia was
infrequently observed by these authors.

Lalezari et al.\textsuperscript{11} reported a family similar to this one. They call the disorder "iso-immune neonatal neutropenia" and consider it to be a condition analogous to erythroblastosis foetalis.

The present study lends support to the studies of other investigators in demonstrating that foeto-maternal leukocyte incompatibility may induce leuko-agglutinin formation in the mother. This study and that of Lalezari illustrate the ability of these leuko-agglutinins to cause severe clinical manifestations, which endanger the life of the offspring.
CHAPTER III

INDUCTION OF LEUKOPENIA IN RABBITS

Regarding antibodies and antibody specificity, Boyd states:

"It has been suggested that the specific combining power of many normal antibodies is due merely to an accidental correspondence between their structure and that of the antigen with which they react, i.e., some arrangement of the various groups on their surface which reflects in reverse that on the antigen. The discovery of the lectins lends credibility to this suggestion." 13

Perhaps leukocytes are not the antigenic stimulus for the formation of leukocyte antibodies, or perhaps leukocyte antibodies are actually antibodies to an antigenic substance other than the leukocyte.

In order to show that leukocytes are antigenic, isolated rabbit leukocytes were injected into other rabbits at suitable intervals, and the injected rabbits were checked periodically for leukocyte antibody formation. While this does not prove that leukocytes are the antigenic substance in all situations where leukocyte antibodies can be demonstrated, it does, however, demonstrate that leukocytes are antigenic in that they can cause the production of leukocyte antibodies.

The activity of the leukocyte immune rabbit serum was investigated by injecting it intravenously into the leukocyte donor rabbits. White blood cell counts were done on the recipient rabbits at intervals to determine whether the immune sera would produce a leukopenic state.
I. MATERIALS AND METHODS

Preparation of antigen. Fiessinger's alcohol-water method was used to isolate leukocytes from whole blood. With this method, 10 ml. of blood were immediately added to 50 ml. of a one-third alcohol, two-thirds water solution, mixed, centrifuged rapidly and the supernatant decanted. The remaining button of cells was pure leukocytes.

Every third day, 20 ml. of blood were taken from rabbit A. The button of leukocytes obtained by using Fiessinger's alcohol-water method was suspended in 4 ml. sterile saline and divided into 1 ml. aliquots for injection.

Preparation of immune rabbit sera. Rabbits number 1, 2, 3 and 4 were each injected intravenously via the marginal ear vein with 1 ml. leukocyte antigen prepared from rabbit A, every third day for three weeks. Blood samples were taken on the seventh day following the last antigen injection, and the sera tested for antibodies using the leuko-agglutinin test. The rabbits which developed significant antibody titres were bled by cardiac puncture using sterile technique. The sera were separated from the clots, frozen and stored. Control sera were taken from rabbits number 1, 2, 3 and 4 previous to any antigen injections.

Immune sera infusion. Sera from the rabbits which showed an immune response, as indicated by the leuko-agglutinin test, were pooled. Forty-five ml. of these immune sera were slowly injected into rabbit A via the marginal ear vein. White blood cell counts
were done on this rabbit at the moment before the infusion was started and at following intervals. A control experiment was carried out one month previous to the infusion of rabbit A with immune sera. This was done by means of intravenous infusions of rabbits A and B with 45 ml. of pooled normal sera from rabbits number 1, 2, 3 and 4. White blood cell counts were done following the infusions. This injection of normal sera was repeated with rabbit B at the time when rabbit A was injected with immune sera.

II. RESULTS

Response to leukocyte antigen injections. Of the group of rabbits which received injections of leukocytes from rabbit A, only rabbits 2 and 3 developed antibodies as indicated by the leuko-agglutinin test. Rabbit 2 sera was positive to a titre of 1:32, while rabbit 3 sera was positive to a titre of 1:16. The normal control sera from rabbits 1, 2, 3 and 4, as well as the sera from rabbits 1 and 4 taken after antigen injections, were negative with leukocytes from rabbit A in the leuko-agglutinin test.

Response to sera infusions. Sera from rabbits 2 and 3 were pooled and used for the immune sera infusion. The white blood cell counts of the infused rabbits are presented in Table I. With infusion of normal sera, the white blood cell counts taken thirty minutes after the infusion was started revealed a marked leukocytosis, which subsided to normal after forty-eight hours. With infusion of immune sera into rabbit A, the fifteen minute count revealed a marked leukopenia, as did the thirty minute, one hour
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and two hour counts. Eight hours following the start of the infusion, the white blood cell count increased, and by forty-eight hours revealed a slight leukocytosis.

III. SUMMARY

The in vivo effects of antileukocyte antibodies have been reported in the following situations: (1) injection of human antileukocytic sera into other humans,\(^2,20,21\) (2) injection of human antileukocytic sera into animals,\(^22,23,24,25\) and (3) injection of either human or animals with antisera prepared in a non-human species.\(^26,27,28,29,30\)

Brittingham\(^31\) found a close and roughly quantitative correlation between leuko-agglutinins in injected blood and the capacity to cause leukopenia in recipients. Also, with the injection of serum, positive for leuko-agglutinins, into man or into animals, the development of leukopenia is usually prompt and precipitous as was seen in this experiment.

Leukopenia was produced in only one rabbit in the present study. While this is not statistically significant, it is, however, in accordance with the findings of other authors.

As shown in Table I, page 19, rabbits A and B both developed a marked leukocytosis following injection of normal sera. Yet, when this same rabbit A was injected with immune sera, it developed a marked leukopenia. If one considers that the only difference between the normal and immune sera is the presence of leuko-agglutinins in the immune sera, then if the leuko-agglutinins
could be removed from the immune sera, one would expect the remaining sera to produce a leukocytosis on infusion. If this is the case, then the leukopenia is even greater than it appears, as the leukocyte antibodies would have to overcome the effect of leukocytosis as well as reducing the normal number of circulating leukocytes.

Naturally occurring antibodies reactive with the leukocytes of other species are no doubt present in the sera of some animals. In order to rule out this class of antibody, only one species was used for this entire experiment. Infusion of normal sera from the same animals, which were later used as a source of immune sera, served as a control in distinguishing between naturally occurring antibodies and acquired antibodies within the same species.
CHAPTER IV

PRODUCTION OF ANTI-HUMAN-LEUKOCYTE ANTIBODIES IN RABBITS

In the previous chapter anti-rabbit leukocyte antibodies were produced in rabbits and in vivo experiments were performed. It would be desirable to carry out these experiments in humans, but the use of human recipients would be unethical and could be dangerous. Therefore, in order to demonstrate the antigenicity of human leukocytes, they were injected into rabbits. The immune response to human leukocytes and to rabbit leukocytes when injected into rabbits were compared.

1. MATERIALS AND METHODS

Preparation of antigen. Two healthy (Group O, Rh-positive) humans were used as leukocyte donors numbered I and II. The donors were bled by veni-puncture. Fiesinger's alcohol-water method 19 was employed to isolate the donor's leukocytes. The isolated leukocytes from 25 ml. of each donor's blood were suspended in 3 ml. sterile saline and divided into one ml. aliquots for injection. Thus, one injection consisted of leukocytes derived from 8.3 ml. of blood.

Preparation of anti-human-leukocyte-immune rabbit serum. Rabbits A, B and C were injected with human leukocytes from donor I. Rabbits D, E and F were used for injections of donor II human
leukocytes. The leukocytes for injection were prepared fresh on the same day as the injections took place. Injections were made with a twenty-five gauge needle, into the marginal ear veins of the rabbits. Each rabbit was injected with one ml. leukocyte antigen, three times weekly, for three weeks. These rabbits were bled seven, fourteen and seventeen days after the last injection. The bloods were allowed to clot, were centrifuged and the sera were removed, inactivated and frozen.

**Leuko-agglutinin test.** All sera were inactivated at fifty-six degrees centigrade for thirty minutes. The immune sera were then absorbed with packed red blood cells from the appropriate donor whose leukocytes had been used for injection. Control sera were obtained from each rabbit prior to the leukocyte injections, and also from each leukocyte donor.

The leuko-agglutinin test as described in Chapter II, page 8, was used to determine the presence of leukocyte antibodies. Sera from rabbits A, B and C were set up against donor I leukocytes. Sera from rabbits D, E and F were set up against donor II leukocytes.

To determine whether the antibodies were specific for the leukocytes of the donor used for injection, sera from rabbits A, B and C were set up against donor II leukocytes, and D, E and F sera against donor I leukocytes. All sera were also set up against human leukocytes from two uninvolved donors numbered III and IV. The immune rabbit sera were also set up against rabbit leukocytes.
II. RESULTS

Sera from rabbits A, B, C, D, E and F, taken fourteen and seventeen days after the last leukocyte injection, gave antibody titres of 1:256 or 1:512 with +++ agglutination up to dilutions of 1:32 or 1:64. The antibody titres of sera taken on the seventh day were two or three dilutions lower when set up against the corresponding donor's leukocytes. All control sera were negative, as were the immune sera set up against rabbit leukocytes.

In the cross agglutination studies, sera A, B, C, against donors II, III and IV, and sera D, E, F, against donors I, III and IV, all gave antibody titres of 1:256 or 1:512.

IV. SUMMARY

The antigenicity of human leukocytes was demonstrated by their ability to cause the production of antibodies in rabbits. The antibodies were not specific for the particular donor's leukocytes which had brought about their production, as the immune rabbit sera caused equally strong agglutination of all donors' leukocytes which were used in this experiment.

Much higher antibody titres were found with the injections of human leukocytes into rabbits than when rabbit leukocytes were injected into rabbits, as noted in the previous chapter.

Brittingham produced leuko-agglutinins to titres of 1:1 to 1:12 by injecting human leukaemic and normal leukocytes into a human recipient. The leuko-agglutinin method which he used
consisted of suspending the leukocytes in albumin rather than saline as was done in these experiments. Therefore the strength of the titres found when human leukocytes were injected into human recipients as was done by Brittingham, when rabbit leukocytes were injected into rabbits, and when human leukocytes were injected into rabbits, cannot be compared.

It was also noted by Brittingham $^{32}$ that the leuko-agglutinin formed by injection of human leukocytes, agglutinated leukocytes of 127 of 129 donors.
CHAPTER V

ANTIGENIC DIFFERENCES BETWEEN POLYMORPHONUCLEAR LEUKOCYTES AND LYMPHOCYTES

The sites of chemical activity which bring antibody and antigen into combination represent relatively small portions of these complex molecules. These small regions of active union are called antigenic determinants on the antigen and specific patches on the antibody. The protein, insulin, illustrates this concept. The difference between beef insulin and pig insulin is known. Of the fifty-one amino acid units in the insulin molecule, forty-eight have the same arrangement in the insulins of different species; the sequence of units in one segment of three units varies. Insulin resistant diabetics recognize this tiny difference between their own insulin and beef insulin and make antibodies against this particular segment.

With regard to these small regions of antigenic determinants and the complex mosaic of proteins, lipids and carbohydrates found in the leukocyte, one could postulate many sites on the leukocyte which could serve as antigenic determinants. The number of possible antigenic determinants is greatly reduced when one considers that the more closely related two species are, the more similar are their proteins and other antigens. Individuals of the same species, however, are not always antigenically exactly alike. An excellent example of this variation is found in the human blood groups.
Leukocyte antigens present an even more complex problem than erythrocyte antigens, as the leukocytes consist of different cell lines and maturation stages. Marchal et al.\textsuperscript{34} state that recipients of multiple injections of even the same donor leukocytes tend to develop a mixture or multiplicity of antibodies reacting with various antigens present in the injected leukocytes.

Antigenic differences and similarities between different types of leukocytes and different morphologic parts of the leukocyte have been reported.\textsuperscript{35,36,37,38,39,40,41,42,43,44} While conflicting views are on record, it does seem tentatively possible to differentiate serologically between all the various types and broad maturation stages of leukocytes, and in a similar manner between normal and leukaemic leukocytes.

Most of the investigations in these aspects of leuko-agglutinins have made use of heterologous antisera. Heterologous systems may be complicated by the presence of cytotoxic factors which are not immune in nature. Species specific antibodies are also apt to be encountered. In order to avoid these complications, a homologous system was used in the following experiment. The object of this experiment was to prepare antisera against polymorphonuclear leukocytes and to observe the effects of these sera on lymphocytes. The polymorphonuclear leukocytes and the lymphocytes were derived from the same animal in order to rule out species or individual specificity.
I. MATERIALS AND METHODS

Preparation of antigen. Rabbit A was used as a source of polymorphonuclear leukocytes. This rabbit was anaesthetized with "pentothal". A polyethylene cannula was inserted into the peritoneum and stitched into place. The free end of the cannula was threaded under the skin to a point on the mid back and the free end brought through a stab wound and stitched in place. With this arrangement the animal was unable to pull out the cannula and injections via the cannula could be made with ease. Two hundred ml. of sterile five per cent saline was injected via the cannula. The cannula was then closed with a sterile plug. Twelve hours later 200 ml. of sterile one per cent saline was injected. After one hour, 50 ml. of peritoneal exudate was removed with a syringe attached to the cannula. This was followed by injection of 100 ml. sterile one per cent saline and the abdomen was kneaded gently. One hundred ml. of exudate were removed. The cannula was removed and the incisions clamped. The samples of exudate were mixed and white blood cell and differential counts were performed. The exudate was then centrifuged, washed three times in sterile saline, suspended in 18 ml. sterile saline, divided into 1 ml. aliquots and frozen.

Preparation of polymorphonuclear antisera. Control blood samples were taken from rabbits number I and II. The blood was allowed to clot, centrifuged, the serum removed and inactivated at fifty-six degrees centigrade for thirty minutes and stored at zero
degrees centigrade.

One ml. of the antigen preparation was injected into rabbits number I and II via the marginal ear vein three times weekly for three weeks. Seven days following the last antigen injection 50 ml. of blood were removed from rabbits number I and II. This blood was allowed to clot, centrifuged and the serum removed. The serum was inactivated and tested by the leuko-agglutinin test for the presence of leukocyte antibodies. The leukocytes for the leuko-agglutinin test were derived from the blood of rabbit A by the usual Dextran sedimentation method.

**Lymphocyte suspension.** Rabbit A, which was used as the source of polymorphonuclear leukocytes, was also used as the lymphocyte source. This rabbit was anaesthetized with "pentothal", its abdomen shaved and a laparotomy incision was made. The splenic vessels were tied off and the spleen removed. The splenic vessels were perfused with saline to wash out the blood. The spleen was cut into small fragments, suspended in 30 ml. saline and shaken in a separatory funnel for fifteen minutes. This suspension was transferred to a large centrifuge tube and allowed to settle for thirty minutes. Ten ml. of supernatant were removed and white blood cell and differential counts were done on it.

**Leuko-agglutinin test.** Sera from rabbits I and II, which were taken before and after injection of the peritoneal exudate of rabbit A, were absorbed with washed erythrocytes from rabbit A. These sera were then set up in the leuko-agglutinin test against the cells derived from the spleen of rabbit A. The test used for
leuko-agglutinins is detailed in Chapter II, page 8.

II. RESULTS

The white blood cell count of the peritoneal exudate from rabbit A was 4,500 per cu.mm. The differential count revealed ninety-eight per cent polymorphonuclear leukocytes and two per cent monocytes.

Sera from rabbits number I and II taken seven days after the last injections of polymorphonuclear leukocytes from rabbit A exhibited leuko-agglutinins with titres of 1:4 and 1:32 respectively when set up with leukocytes from rabbit A. Control sera from rabbits I and II taken prior to injections gave negative results with the leuko-agglutinin test.

The white blood cell count of the splenic preparation was 5,050 per cu.mm. The differential count revealed 100 per cent lymphocytes.

The leuko-agglutinin test utilizing this lymphocyte preparation and the immune sera from rabbits I and II (taken after the injections of polymorphonuclear leukocytes) were all negative as were the controls.

III. SUMMARY

The antisera produced in rabbits against rabbit polymorphonuclear leukocytes did not produce in vitro agglutination of lymphocytes. This same antiserum did produce in vitro agglutination of the leukocytes separated from rabbit blood. The same rabbit was
used as a source of polymorphonuclear leukocytes for injection, of lymphocytes, and of leukocyte suspensions from blood.

These findings are in agreement with those of Finch et al. who reported that antigranulocytic sera agglutinated human leukaemic granulocytes, but not lymphocytes. Finch also found that antilymphocytic sera produced slight agglutination of both leukaemic granulocytes and lymphocytes. The investigations of Finch differed from the present study in that he used antisera prepared in rabbits against human granulocytes; the present study utilized antisera prepared in rabbits against rabbit granulocytes in order to avoid species specific antibodies.
CHAPTER VI

EFFECT OF SPECIFIC ANTIBODY UPON PHAGOCYTIC ACTIVITY OF HUMAN LEUKOCYTES

Microcinematography plus phase contrast microscopy now allows a very detailed analysis of all movements of the cell and of movements of intracellular material to be made. It also makes possible an analysis of the way in which the morphology and the dynamics of even the finest structures are affected by different media or different forms of injury.

By means of microcinematography and phase contrast, Andre et al. observed human leukocytes exposed to the leuko-agglutinating serum of a patient who had received over four hundred transfusions for chronic idiopathic pancytopenia. The leukocytes underwent nuclear changes consisting of oedema, distention to a spherical form, homogenization and increasing pallor of chromatin, and finally lysis. Cytoplasmic alterations were variable.

Bessis and Bessis and Fabins also investigated the cytologic changes induced in human leukocytes by antileukocytic serum. The changes associated with agglutination, lysis and phagocytosis were variable.

Miescher and Fauconnet compared the cytologic changes induced in leukocytes by anticytoplasmic and antinucleoprotein sera. The former led to cytoplasmic vacuolization and lysis and to nuclear lysis. In the presence of the latter, the nuclei lost
their staining quality and became granular. Damaged cell nuclei were phagocytosed by intact leukocytes.

From these studies which utilized phase contrast and microcinematography it was shown that antileukocytic sera caused cytopathologic changes in leukocytes. The end result was usually cell death, although in some cases the cells were considered to be alive but morphologically altered. The following experiment was set up to determine if these morphologically altered cells were still capable of their prime function, i.e. phagocytosis.

Granulocytes and particularly neutrophils, have the ability to ingest and to digest microorganisms and foreign particles. The importance of this defense mechanism is aptly illustrated in leukopenic states where the patient is unable to resist infections ordinarily attended by leukocytosis.

I. MATERIALS AND METHODS

Anti-human-leukocyte rabbit serum was tested for phagocytosis-inhibition. Normal rabbit serum and normal human serum were used as controls. The same donor was used as a source of leukocytes for the test and as a source of leukocytes for preparation of the anti-human-leukocyte rabbit serum.

All sera were inactivated at fifty-six degrees centigrade for thirty minutes and diluted 1:4 with sterile saline. The human leukocyte suspension was prepared by the Dextran sedimentation method. The leukocytes were concentrated to about 10,000 per cu.mm.
One-tenth ml. of the diluted test serum was added to 0.5 ml. leukocyte suspension and was incubated for one-half hour at thirty-seven degrees centigrade. A few drops of a slightly opaque suspension of heat-killed staphylococci was then added to each tube. The tubes were incubated one-half hour. Gram stained smears were made of each specimen and one hundred neutrophils were examined microscopically and the average number of cocci per phagocytic cell was determined.

II. RESULTS

Serum from normal rabbit A, normal rabbit B, and the human leukocyte donor gave values of 75 per cent, 88 per cent and 84 per cent respectively for the per cent of neutrophils containing phagocytosed cocci. Anti-human-leukocyte immune rabbit serum A and B gave values of 68 per cent and 74 per cent respectively for the per cent of neutrophils containing phagocytosed cocci.

When the leukocytes were incubated with normal rabbit A serum, normal rabbit B serum and the human leukocyte donor serum, the average number of cocci per phagocytic cell was 6.1, 4.9 and 5.7 respectively. With anti-human-leukocyte immune rabbit serum A and B, the average number of cocci per phagocytic cell was 0.92 for the former and 1.5 for the latter.

III. SUMMARY

The percentage of neutrophils containing phagocytosed cocci was not appreciably affected by prior incubation with anti-human
leukocyte immune rabbit sera A and B.

The number of cocci per phagocytic cell was markedly less in the cells exposed to the immune rabbit sera than in the cells exposed to the normal rabbit and human leukocyte donor sera.

Walford states that the phagocytosis-inhibition test in a homologous human system probably parallels the leuko-agglutinin test in most instances. Matoth et al., however, described a leuko-agglutinating serum that did not inhibit leukocyte phagocytic activity for starch particles.

Stollerman says: "Diminished phagocytosis in a particular instance might be due to a defect in the phagocytosis-accelerating system of human plasma rather than to a specific antibody." It was noted by Finch and Detre that physiologic amounts of chloramphenicol, PAS and versene would depress phagocytic activity of leukocytes. Although in this experiment a correlation was found between decreased phagocytosis and antileukocytic immune sera, non-immune factors must be borne in mind and one cannot conclude that the decreased phagocytosis is caused by the specific antibody which brings about leuko-agglutination. The presence of two distinct entities is suggested by Surgenor et al. By electrophoresis and fractronation they found activity distributed into two discrete fractions. Tests on the separated components revealed that the slow component tended to exhibit lytic and agglutinating activity. The fast component seemed to be more potent in decreasing viability of the test cells. Moreover, the fast component differed from the slow component in stability and in other physical properties. In
none of the sera they tested did activity appear in the gamma globulin fraction. A number of other investigators 55,56,57,58,59 have located leuko-agglutinins chiefly in the gamma globulin fractions. There is currently no explanation of these somewhat variable results from different laboratories, except the simple one that antibodies are usually in, but not necessarily restricted to, the gamma globulin component of serum.
CHAPTER VII

INVESTIGATION OF A VARIETY OF LEUKOPENIC STATES

Leukopenia is a reduction in the number of leukocytes in the peripheral blood below 4,000 per cu.mm. In some instances leukopenia is caused by a reduction in all types of leukocytes. Usually the leukopenia is caused by a reduction of only one type of leukocyte, in which case it can be more exactly defined as neutropenia, lymphopenia, eosinopenia, etc. Neutropenia is a reduction in neutrophil leukocytes below 1,500 per cu.mm. in children, and below 1,800 per cu.mm. in adults. In the present connection the neutrophils are of outstanding importance because leukopenia is virtually synonymous with neutropenia. Lymphopenia occurs rarely and in practical haematology is most frequently the result of radiotherapy.

Ex hypothesi neutropenia or any other cytopenia might be brought about by defective formation of the cells in question, by their excessive destruction, or by some impediment to their emigration from the bone marrow. Little practical help is gained by attempting to compress the causes of neutropenia into these categories, because in many instances the operative mechanism is not understood.

During recent years leukocyte agglutinins have been suspect as a cause of some leukopenias. Following the demonstration by Moeschlin of leuko-agglutinins in agranulocytosis due to drugs, and
of leuko-agglutinins in idiopathic leukopenia by Dausset and Nenna, a number of reports appeared on leukopenia associated with leuko-agglutinins. It was suggested that these leukopenias were due to an auto-antibody action of the leukocyte agglutinins demonstrated. The actual clinical significance of the finding of leuko-agglutinins, i.e. the question as to whether or not these human antibodies are autoaggressive in vivo or whether they are a mere epiphenomenon, has not been clarified.

The present investigation was undertaken in an attempt to correlate leukocyte agglutinins with leukopenia. Sera from leukopenic patients were collected and stored over a period of several months. By the time that the study was completed, a diagnosis had been reached for many of the patients and in several cases leukopenia no longer existed.

I. MATERIALS AND METHODS

The patients were all admitted to one hospital so that laboratory methods and diagnostic criteria used were uniform. Complete histories were taken which included the number of transfusions and the drugs the patients had received.

A synoptic presentation of each case history is included in the Appendix page 60.

Preparation of the leukocyte suspensions. Eight healthy (Group O, Rh positive or negative) individuals were used as blood donors. Leukocyte suspensions were prepared from the blood of these donors by Dextran sedimentation followed by resedimentation
in saline, as was outlined in Chapter II, page 7.

**Preparation of sera.** All patients' and control sera were heat inactivated at fifty-six degrees centigrade for thirty minutes. Sera from two normal individuals were used as negative controls. Anti-human-leukocyte immune rabbit serum was used for a positive control.

**Leuko-agglutinin test.** The same method was used as cited in Chapter II, page 8. Each serum was set up against the panel of leukocyte suspensions. The positive and negative control sera were set up with each leukocyte suspension every time a patient's serum was tested.

**Addition of drug to the test serum.** Because cases number 9 and 14 had been on chloromycetin therapy previous to their leukopenic states, and because of the association of this drug and leukopenia, the drug was added to the patient's serum to determine whether it would augment or restore leuko-agglutinating activity. One-tenth mg. chloromycetin per ml. of serum was incubated with the patients' sera and a normal serum overnight at four degrees centigrade. These incubated sera were set up in the leuko-agglutinin test against two leukocyte suspensions. Serum from each patient was set up with a leukocyte suspension which showed no agglutination when previously set up in the absence of the drug, and also with a leukocyte suspension which showed agglutination in the absence of the drug. A restoration of leuko-agglutinating activity was looked for with the former leukocyte suspension, and an augmentation of the leuko-agglutinating activity with the latter.
II. RESULTS

The positive control serum, i.e. the anti-human-leukocyte immune rabbit serum, exhibited strong agglutination with the entire panel of leukocyte suspensions. Serum from a patient was not considered positive for leuko-agglutinins unless the results were reproducible and unless the leukocytes were devoid of any clumping in the two negative controls.

The results of the seventeen patients' sera surveyed during the course of this study are reported in Table II. All the patients except one had received transfusions. On completion of the study many of the patients had been diagnosed as having malignant disease. As shown in Table II, only serum from case number 4 showed agglutination of the entire panel of leukocyte suspensions. It should be pointed out, moreover, that some leukocyte suspensions which gave a negative reaction with the serum from one case gave a positive reaction with serum of another case and vice versa. A considerable variation in the titre of agglutination with the various leukocyte suspensions exposed to the same patient's serum was also noted. In this limited study there appears to be no correlation between the final diagnosis and the presence of leuko-agglutinins, or the severity of the leukopenia and the strength of the leuko-agglutinin.

The addition of chloromycetin to the serum of the patients previously treated with it had no effect whatsoever.
Table II - see over
<table>
<thead>
<tr>
<th>Case No. &amp; Sex</th>
<th>Blood Cell Levels/cu. mm.</th>
<th>Final Diagnosis</th>
<th>No. of Transfusions</th>
<th>No. of W.B.C. Suspensions Tested</th>
<th>No. with Pos. Leukoaggl. Reactions</th>
<th>Extremes of Pos. Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 61 F.</td>
<td>3,500 - 191,500</td>
<td>Chronic lymphatic leukaemia</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2. 20 M.</td>
<td>3,050 - 8,450</td>
<td>Idiopathic pancytopenia</td>
<td>27</td>
<td>8</td>
<td>1</td>
<td>(+) to ++</td>
</tr>
<tr>
<td>3. 69 M.</td>
<td>1,600</td>
<td>Intermittent neutropenia</td>
<td>25</td>
<td>8</td>
<td>4</td>
<td>(+) to ++</td>
</tr>
<tr>
<td>4. 84 F.</td>
<td>2,850</td>
<td>Chronic idiopathic pancytopenia</td>
<td>over 50</td>
<td>8</td>
<td>8</td>
<td>(+) to +++</td>
</tr>
<tr>
<td>5. 17 M.</td>
<td>3,000</td>
<td>Idiopathic pancytopenia</td>
<td>over 150</td>
<td>8</td>
<td>4</td>
<td>(+) to ++</td>
</tr>
<tr>
<td>6. 65 F.</td>
<td>400 - 3,000</td>
<td>Neutropenia and anaemia</td>
<td>over 50</td>
<td>8</td>
<td>3</td>
<td>(+) to +++</td>
</tr>
<tr>
<td>7. 80 M.</td>
<td>1,400 - 1,700</td>
<td>Multiple myeloma</td>
<td>over 14</td>
<td>6</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>8. 68 M.</td>
<td>400 - 1,800</td>
<td>Acute aleukaemic leukaemia</td>
<td>over 12</td>
<td>8</td>
<td>6</td>
<td>+ to ++</td>
</tr>
<tr>
<td>9. 54 F.</td>
<td>800</td>
<td>Pancytopenia, aplasia</td>
<td>over 40</td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Age</td>
<td>Sex</td>
<td>Count</td>
<td>Description</td>
<td>Value</td>
<td>Symptoms</td>
</tr>
<tr>
<td>-----</td>
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</tr>
<tr>
<td>10.</td>
<td>18 F.</td>
<td>1,050</td>
<td>Chronic lymphatic leukaemia over 50</td>
<td>5</td>
<td>2</td>
<td>+ to ++</td>
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<td>11.</td>
<td>74 M.</td>
<td>2,200 - 3,100</td>
<td>Aplastic anaemia, agranulocytosis</td>
<td>6</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>12.</td>
<td>M.</td>
<td>7,350</td>
<td>Idiopathic neutropenia</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>13.</td>
<td>76 F.</td>
<td>250 - 9,200</td>
<td>Erythraemic myelosis</td>
<td>25</td>
<td>8</td>
<td>4 (++)</td>
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<td>14.</td>
<td>75 M.</td>
<td>2,100 - 17,200</td>
<td>Acute myelogenous leukaemia over 25</td>
<td>8</td>
<td>2</td>
<td>++</td>
</tr>
<tr>
<td>15.</td>
<td>74 M.</td>
<td>1,700 - 2,200</td>
<td>Acute aleukaemic monocytic leukaemia</td>
<td>15</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>16.</td>
<td>1 M.</td>
<td>10,250</td>
<td>Possible aplastic anaemia, lymphocytosis, granulocytopenia</td>
<td>2</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>17.</td>
<td>32 F.</td>
<td>3,000 - 5,000</td>
<td>Idiopathic neutropenia</td>
<td>5</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>
III. SUMMARY

Obviously, if leukocyte antibodies were found in normal individuals without prior transfusion, the finding of them in various blood dyscrasias would be meaningless. Thus it is pertinent to review the studies on normal sera. Dausset found negative leuko-agglutinin reactions with the sera of at least two thousand normal individuals, Bagdasarov et al. with five hundred, and Andre et al. with two hundred normal sera. A few other studies have been either negative or shown weak or irregular reactions.

In this study all patients except one had received multiple transfusions. Payne, in a large study, concluded that the supposed association of leuko-agglutinins with neutropenia was probably specious, and that a history of multiple transfusions was in fact the principle denominator. Andre et al. and Brittingham reached the same conclusion.

Leuko-agglutinins, however, have been found in a minority of cases, in the absence of a history of transfusions. Eyquem et al. noted leuko-agglutinins in fifteen of a total of one hundred and seventy-five cases of chronic rheumatoid disease. Killmann observed that four out of eighteen leuko-agglutinating sera of various collagen diseases were from untransfused patients without previous pregnancy.

The importance of blood transfusions in stimulating the production of most leuko-agglutinins is established beyond all doubt,
Dausset claims. Nevertheless, not all polytransfused patients produce leuko-agglutinins.

In this study, before a correlation between the leukopenic state, the underlying disease, and the leukocyte antibodies could be made, it would have been necessary to obtain samples of sera previous to any transfusions. This was not possible.

As regards drug-induced immune leuko-agglutination, Walford states that in most cases of drug-induced immuno-neutropenia the leuko-agglutinating potency is not increased by adding the drug to the serum. There have been a few cases reported, however, where the phenomena could be potentiated by adding a small quantity of the drug in question to the test system. The suspected drug, chloromycetin, in the two cases reported in this study, had no effect on the leuko-agglutinating ability of the patients' sera. In view of these results and the above statements, one cannot conclude whether chloromycetin was responsible for the leukopenic states of the patients or not.
CHAPTER VIII

DISCUSSION

The scope of this study is too limited to warrant broad generalizations on the subject of leukocyte antibodies. Nevertheless, certain facts were established which are in harmony with the findings of other investigators in this field.

One of the more important findings in this investigation was the role played by leukocyte antibodies in leukopenia of the newborn, analogous to the anaemias of erythroblastosis. Although Payne and Rolfs 17 found leuko-agglutinins in the sera of twenty-five of one hundred and forty-four pregnant multiparous women, no effect on the newborn of maternal leukocyte iso-agglutinins was reported. In this study three of the six offspring of one family were severely neutropenic and maternal iso-immune antibodies were demonstrated. Thus the situation involving the white blood cells, analogous to erythroblastosis foetalis, is a real and important entity but appears to be rarely encountered.

The experimental production of leukocyte antibodies by intravenous injections of homologous leukocytes demonstrates the antigenic capacity of the leukocyte. The infusion of leukocyte immune sera causes a transient neutropenia as was reported with rabbits in these experiments and with humans by Brittingham. 32 While these phenomena explain the presence of leukocyte antibodies in polytransfused patients and the occurrence of febrile transfusion reactions in
patients with leukocyte antibodies, they do not account for the presence of leuko-agglutinins found in certain pathological cases with negative transfusion histories.

Leukocyte antibodies have been demonstrated in a number of disease states, such as leukaemia, Hodgkin's disease, rheumatoid diseases, Felty's syndrome, idiopathic pancytopenia and leukopenia, agranulocytosis, cyclic neutropenia, acquired haemolytic anaemia, idiopathic thrombocytopenic purpura, paroxysmal nocturnal haemoglobinuria and drug induced leukopenia. A judgement cannot be made as to whether a compilation of these diseases, in which leukocyte antibodies have been demonstrated, has any basic significance or merely reflects those entities in which a large number of transfusions are usually given. Leukocyte antibodies are not always found in these disease states, regardless of transfusions, so their presence gives no aid in establishing a diagnosis.

Payne 69 found no relationship between leuko-agglutinins and the diagnosis. The development of the antibodies appeared entirely and simply related to the number of transfusions received. Dausset 70,71 suggested an association between neutropenia and leuko-agglutinin formation. He found neutropenia in eighty-five per cent of his large series of leuko-agglutinating sera, but his data on this particular point may be criticized for not representing a random sampling of patient sera, as two hundred and eighty of the six hundred and thirty patients selected for testing were leukopenic. Patients selected for the present study were all leukopenic at the time of investigation. Of these seventeen leukopenic sera, six
exhibited no agglutination of the panel of leukocytes. Only one of the eleven positive sera agglutinated the entire panel.

The negative results and the very low titres in some of the cases may be due to treatment. For example, case number 2 was diagnosed as idiopathic pancytopenia and had received over twenty-seven transfusions. Thus he would seem a likely candidate for leukocyte antibodies. His sera only weakly agglutinated one leukocyte suspension of the panel of eight. This patient had previously been on steroids and also had a splenectomy, which may account for the apparent absence of, or very weak, leukocyte antibodies.

The significance of leuko-agglutinins in polytransfused patients lies in their frequent association with febrile transfusion reactions. These may be quite severe. They can be prevented by employing leukocyte poor blood. The severity of the clinical reaction depends on the dose of transfused leukocytes and the strength of the recipient's leuko-agglutinins. 32

While the finding of leukocyte antibodies in various disease states is of considerable theoretical interest and has a certain degree of practical importance, in some transfusion reactions, the demonstration that these leukocyte antibodies are actually directed against the patient's own leukocytes, i.e. are auto-antibodies, is paramount. The main obstacle in the investigation of auto-antibodies in suspected cases is the danger to the patient of removing a sufficient quantity of blood to be used as the source of leukocytes for the test. The patients are often leukopenic and
anaemic to begin with, and are therefore not a good source of leukocytes. These difficulties were encountered in the present investigation, thus auto-antibodies were not studied.

Some inferential evidence favouring the existence of leukocyte auto-agglutinins can be found in the literature, but it is becoming increasingly clear that the great majority of leuko-agglutinins are in fact simply iso-antibodies, rather than auto-antibodies. However, the possibility exists that the techniques for demonstrating leukocyte antibodies are not sufficiently delicate or sensitive to demonstrate auto-antibodies.

Regarding the clinical significance of leukocyte antibodies, all that can be said on present data with respect to the genesis of leukocyte antibodies in man is that they tend to develop in patients with haematologic disease, usually not until multiple transfusions have been given, that many of these patients have associated neutropenia, and that the relationship of the neutropenia to the general process is wholly unknown.

Walford 72 states that one, and doubtless multiple, antigens are present in the human leukocyte. Seligmann and his associates 73,74 demonstrated the existence of a variable number of antigens in human leukocytes. This study demonstrated that serum with leuko-agglutinins obtained from multiple transfused patients may not react with all tested leukocytes and that different leukocytes react differently with a panel of leuko-agglutinating sera, suggesting that a classification of leukocyte "types" may be possible. Lalezari et al. 75 found that leukocytes of identical
twins presented identical reaction patterns when tested against leuko-agglutinating sera. He concluded that there is a multiplicity of leukocyte antigens and that these are not related to erythrocyte antigens.

Accurate serologic typing of white blood cells would require adsorption with packed leukocytes in order to remove all but one type of antibody. This is generally not feasible, since most leuko-agglutinins are of low titre and nonspecific adsorption would reduce the titre to a level unworkable for accurate comparative studies.

Knowledge of leukocyte immunohaematology may have some bearing on the problems of tissue transplantation. Antibodies reacting with leukocytes can in certain instances be demonstrated following homologous tissue transplantation of either normal tissue such as skin, or of neoplasms. The transfusion of white blood cells can legitimately be regarded as a type of homograft. Transplantation antigens, i.e. iso-antigens responsible for homograft rejection, are not tissue specific, but shared in common by all living cells of a single individual. Leukocytes, skin, spleen, testis and doubtless other organs share these common antigens within the individual. On the other hand, red blood cells, for reasons not yet understood, behave as if they contained no transplantation antigens; yet the important and familiar iso-haemagglutinins they do possess are also distributed widely in tissue cells and are closely related in a genetic sense. White blood cells differ from red blood cells in this respect, as they do
possess transplantation antigens. Evidence of this is found in skin homografting, where prior injections of white blood cells from a particular donor into a recipient leads to accelerated breakdown of skin that is subsequently homografted from the same donor to the same recipient, i.e. it leads to a type of "second-set phenomenon" in which there is accelerated breakdown of a tissue graft in an animal who has received and rejected a previous graft from the same donor. 77

Billingham 78 found that if cellular homografts were enclosed in porous chambers made with membranes of different pore diameters and inserted in the body cavities of specifically sensitized animals they will be destroyed only if the holes are large enough to admit host leukocytes. Billingham states, "It is conceivable that "cell-bound" antibodies transported by these activated lymphocytes are the destructive agents." 78

The only hopeful approach to a solution of the homograft problem entails trying to devise a treatment for an intended host that will make it specifically incapable of reacting against the transplantation antigens of an intended donor. This solution has been partially met by giving a sufficiently high dosage of X-irradiation to the graft recipient, so that both the lymphoid tissue and bone marrow are destroyed. Bone marrow transplants are then given to the graft recipient from the graft donor.

An interesting speculation can be made from the above knowledge. Perhaps leukocyte immune sera specific for the graft recipient's lymphocytes could be infused into the graft recipient to
destroy his leukocytes until such time as the graft had taken. This would also circumvent the wasting disease, found in radiation chimeras rehabilitated with homologous bone marrow, attributed to the immunological activity of lymphoid cells included in the transfused marrow.

Many other speculations can be made regarding leukocyte antibodies. Perhaps antileukaemic antibodies could be used in the treatment of some leukaemias.

Apart from the role of leukocyte antibodies in immunohaematology, this subject may have an even more rewarding future in elucidating the mechanisms of auto-immune disease phenomena and of the homograft reaction. The leukocyte is well adapted to inquiries of this sort. They can be harvested in a form reasonably amenable to serologic analysis, not only with regard to classic agglutination, lysis, complement fixation and precipitin techniques, but also with regard to their well established and easily studied functional activities, such as motility, phagocytosis, vital staining and other properties.
BIBLIOGRAPHY


51. Walford, op. cit., p. 25.


Aspects serologiques et serologie des leucocytes en

57. Martensson, J., and Vikbladh, I.: Idiopathic immunoneutropenia.
Report of a case with a leukocyte agglutinating factor in

58. Saathoff, J., and Kuesel, H.: Leukozytenagglutination bei
Pyramidonagranulocytose, Lupus Erythematodes und einem

sandinav. 45: 12, 1959.

60. Miaele, J.B.: Laboratory Medicine - Hematology. St. Louis,

61. Dausset, J., and Maupin, B.: Indications des derives du sang
dans le traitement des agranulocytoses. Sang 27: 20,
1956.


63. Bagdasarov, A.A., and Dvolaitskaia-Barysheva, K.M.: Anti-
leucocyte antibodies in hypoplastic anemia and chronic
217, 1958.

64. Andre, R., Dreyfus, B., and Bessis, M.: Iso-immunization anti-
leucocytes apres transfusions. Haematologica 42: 1223,
1957.

between blood transfusions and their development. Arch.

leuco-agglutinines au cours des rhumatismes chroniques
soumis a des therapeutiques antiinflammatoires. Sem.
1955.


CASE HISTORIES

CASE 1: The patient was a sixty-one year old female with a twelve year history of chronic lymphatic leukaemia. Three years ago she was treated with total body irradiation. Subsequently thrombocytopenia was treated with Cortisone followed by Chlorambucil. Thrombocytopenia persisted, but megakaryocytes were plentiful in the bone marrow. Therefore, a splenectomy was done and the platelet count rose to normal and has remained in normal range ever since. During the past twelve years her white blood cell count ranged from 3,500 per cu.mm. to 191,500 per cu.mm., with 2% to 24% neutrophils and 53% to 90% lymphocytes. Apart from two attacks of pneumonia the patient has been very well with an excellent remission.

CASE 2: The patient was a twenty year old male with a two year history of nosebleeds, fatigue, petechiae and anaemia. Laboratory data within the past two years fell within the following ranges: haemoglobin 7.4 to 11.9 Gm. per 100 ml.; white blood cell count 3,050 to 8,450 per cu.mm. with differential count of 19% segmented, 12% bands, 59% lymphocytes, 2% monocytes and 5% disintegrated cells; platelets 8,280 to 37,680 per cu.mm. Chromium 51 studies revealed a red cell life span of 6.5 days instead of the normal 30 days. Bone marrow aspiration revealed marked hyperplasia with proliferation of the erythropoietic and granulopoietic cells. A splenectomy was performed with no resulting change in the haematological picture. The patient was tried on steroids, but failed to improve. The patient had received multiple transfusions in the past two years. Clinical diagnosis: idiopathic pancytopenia.

CASE 3: The patient was a sixty-nine year old male with a two year history of intermittent neutropenia, characterized by a decline of peripheral leukocytes to 1,600 per cu.mm. The haemoglobin was 10 Gm. per 100 ml. The bone marrow was hypercellular with mild immaturity of both the myeloid and erythroid series. The Coombs' test and L.E. test were negative. The patient did not respond to steroids. The patient had received twenty-five transfusions in the past. Clinical diagnosis: intermittent neutropenia and anaemia.

CASE 4: The patient was an eighty-four year old woman. A typical blood picture was: haemoglobin 6.9 Gm. per 100 ml.; white blood cell count 2,850 per cu.mm. with differential count of 44% neutrophils, 37% lymphocytes and 19% monocytes; platelets 58,170 per cu.mm.; reticulocytes 4% to 7%. The Coombs' test was negative. The bone marrow aspiration showed marked hyperplasia of erythrocyte, granulocyte and platelet precursors, and haemosiderosis was reported. The patient had received over fifty transfusions in the past years. A previous urinary infection was
treated with sulfas, Chloramphenicol and Mandelamine. Other drugs received were Vitamin B12, steroids and folic acid. The patient was seen by the Cancer Clinic at the University Hospital in Saskatoon and discharged as "non-malignant". Clinical diagnosis: chronic idiopathic pancytopenia.

CASE 5: The patient was a seventeen year old male with a past history of fatigue, anaemia and a mild bleeding tendency. He had had a splenectomy five years ago. A typical blood picture was: haemoglobin 4.8 Gm. per 100 ml.; white blood count 3,000 per cu.mm., with differential count of 18% segmented, 9% bands, 72% lymphocytes and 1% monocytes; platelets 10,900 per cu.mm.; reticulocytes 0.2%. The patient had received steroids, antibiotics and over one hundred and fifty transfusions. Clinical diagnosis: idiopathic pancytopenia.

CASE 6: The patient was a sixty-five year old male with a six year history of neutropenia. He had received over fifty transfusions for anaemia, and various antibiotics for infections. The patient had hepatomegaly and splenomegaly with myeloid metaplasia and extra medullary haemopoiesis. Laboratory data during the past year fell within the following ranges: haemoglobin 7 to 12.5 Gm. per 100 ml.; white blood cell count 400 to 3,000 per cu.mm., with differential count of 0 to 4% neutrophils; platelets 145,256 per cu.mm. The score of the alkaline phosphatase stained blood film was 321, which was against the diagnosis of leukaemia. Past illness were; diverticulitis with rupture and a barium sulphate peritonitis, large bowel obstruction, paralytic ileus, ventral hernia, acute maxillary sinusitis, bronchopneumonia, cystitis, fractured femur and possible cirrhosis of the liver. The haematological diagnosis was: neutropenia and anaemia, etiology undetermined.

CASE 7: The patient was an eighty year old man with a past history of jaundice, anaemia, leukopenia and prostatic hypertrophy. His haemoglobin ranged from 4.6 to 7.1 Gm. per 100 ml. A typical white blood cell count was 1,700 per cu.mm. with a differential count of 49% segmented, 1% bands, 47% lymphocytes and 3% monocytes. He remained undiagnosed for two years. A differential count after this time revealed 82% lymphocytes and 3% unidentified cells in the peripheral blood, and 51.5% proplasmacytes and 6.2% plasmablasts in the bone marrow. Diagnosis: multiple myeloma.

CASE 8: The patient was a sixty-eight year old male and presented with a Kaposi cell sarcoma of the leg. Laboratory data was: haemoglobin 5.5 Gm. per 100 ml.; white blood cell count 1,800 per cu.mm., with differential count of 10% neutrophils, 89% lymphocytes and 1% monocytes; platelets 37,610 per cu.mm.; bone marrow 9.2% lymphoblasts, 22.2% prolymphocytes and 55.8% lymphocytes. The patient was treated with steroids, numerous
antibiotics, 6-mercaptopurine and over twelve transfusions. Diagnosis: acute aleukaemic lymphatic leukaemia.

CASE 9: The patient was a fifty-four year old female with a one year history of pancytopenia following a prolonged excessive self-administration of Chloramphenicol. The haemoglobin was 7.5 Gm. per 100 ml. The white blood cell count was 800 per cu.mm., with a differential count of 100% lymphocytes. No platelets were seen. The bone marrow aspiration showed marked atrophy of all haemopoietic cells. The patient received over forty transfusions. Autopsy and clinical diagnosis: septic ulceration of the small and large intestine and pancytopenia.

CASE 10: The patient was an eighteen year old female with a clinical history of pancytopenia for the past six years. On admission the haemoglobin was 7.6 Gm. per 100 ml.; the white blood cell count was 1,050 per cu.mm., with a differential of 66% lymphocytes, 24% prolymphocytes and 10% disintegrated cells. The platelet count was 60,300 per cu.mm. The patient received over fifty transfusions, prednisone and antibiotics. The bone marrow aspiration revealed 12.4% lymphoblasts, 40.4% prolymphocytes, 23.4% lymphocytes. Autopsy and clinical diagnosis: chronic lymphatic leukaemia and lymphatic leukaemic infiltration of the viscera and bone marrow.

CASE 11: The patient was a seventy-four year old male. Laboratory data during the last year fell within the following range: haemoglobin 7.6 - 12.8 Gm. per 100 ml.; white blood cell count 2,200 to 3,100 per cu.mm., with a differential count of 1% neutrophils, 9% eosinophils, 66% lymphocytes, 15% monocytes, 6% basophils and 3% disintegrated cells; platelets 145,140 to 332,000 per cu.mm.; osmotic fragility of 0.50 - 0.38%. The bone marrow aspiration showed atrophy of haemopoietic bone marrow with focal hyperplasia. The patient had received six transfusions. Clinical diagnosis: acute myocardial infarction, early aplastic anaemia with agranulocytosis, and duodenal diverticuli. The patient was reviewed after three months and the bone marrow showed slight hyperplasia.

CASE 12: The patient was a three month old male baby. The child was well from birth to two months of age. The mother was twenty-two years of age and was healthy. On admission the haemoglobin was 11.4 Gm. per 100 ml.; white blood cell count was 7,350 per cu.mm., with a differential count of 8% neutrophils, 1% eosinophils, 85% lymphocytes, 1% prolymphocytes and 5% monocytes. The platelet count was 307,500 per cu.mm. The bone marrow aspiration showed moderate hyperplasia, with 14.2% segmented, 27.6% bands, 7.6% metamyelocytes, 6% myelocytes, 4.8% progranulocytes, 0.6% myeloblasts, 1.8% eosinophils, 0.2% basophils, 25.2% lymphocytes, 0.8% monocytes and 10.2% erythrocyte precursors. Sera from the mother and baby showed agglutination of the father's leukocytes.
The baby's serum was set up against the panel of leukocytes, as seen in Table II. Diagnosis: neutropenia of undetermined etiology.

CASE 13: The patient was a seventy-six year old female with a past history of polycythaemia rubra vera, with a haemoglobin of 19.6 Gm. per 100 ml., haematocrit of 74%, white blood cell count of 9,200 per cu.mm., with differential count of 58% segmented, 16% bands, 4% eosinophils, 1% basophils and 21% lymphocytes. She was treated with 19.5 millicuries of P32 over a period of fifty-four months. The next phase of her illness was characterized by a neutropenia with an absolute lymphocytosis. White blood cell count was 950 to 1,450 per cu.mm. with a differential count of 7% neutrophils, 89% lymphocytes and 4% prolymphocytes. The haemoglobin at this time was 10.2 Gm. per 100 ml. and the haematocrit was 30%. Five months later the differential count of the peripheral blood revealed 14% unidentified cells. Bone marrow aspiration showed 57.4% atypical rubriblasts. The haemoglobin fell to 4.4 Gm. per 100 ml. The patient had received twenty-five transfusions. Autopsy and clinical diagnosis: erythraemic myelosis of haemopoietic bone marrow, erythraemic infiltration of the lymph nodes.

CASE 14: The patient was a seventy-five year old male who's health began to deteriorate five years ago with bouts of chills, fever, dysuria, urinary frequency and a mild haemolytic anaemia. The white blood cell count was 7,200 per cu.mm. with a normal differential. The bone marrow showed marked hyperplasia and haemosiderosis. The diagnosis of mild acquired haemolytic anaemia was made, the patient was treated with Prednisone and discharged as non-malignant. Four years later he had an acute attack of pyelonephritis and was treated with Chloromycetin and Declomycin. Following this therapy the white blood cell count ranged from 2,100 to 17,200 per cu.mm., with a differential count of 1% segmented, 4% bands, 19% lymphocytes, 46% unidentified pro cells, 30% unidentified blast cells. The bone marrow aspiration revealed 46.4% unidentified pro cells and 28.8% unidentified blast cells. These cells were peroxidase positive and Auer bodies were seen. The patient received 6-mercaptopurine, steroids and over twenty-five transfusions. Autopsy and clinical diagnosis: acute myelogenous leukaemia and pyelonephritis.

CASE 15: The patient was a seventy-four year old man with a one month history of blurring of vision, fatigue and purpura. The white blood cell count was 1,700 per cu.mm. with a differential count of 12% segmented, 12% bands, 6% metamyelocytes, 1% myelocytes, 2% progranulocytes, 42% lymphocytes, 5% monocytes, 1% eosinophils, 17% unidentified pro cells and 2% unidentified blast cells. The platelet count was 9,340 per cu.mm. The patient was treated with fifteen transfusions, steroids and antibiotics. Bone marrow aspiration revealed invasion of fatty tissue by neoplastic cells.
with 72.5% unidentified blasts and 6% unidentified pro cells. A diagnosis of acute leukaemia and pancytopenia was made. The patient died of heart failure, acute pulmonary oedema and massive haemorrhages. Autopsy and clinical diagnosis: acute aleukaemic monocytic leukaemia.

CASE 16: The patient was a one year old boy. Three months after birth he developed fever, chills, diarrhoea. He was treated with penicillin for gastroenteritis. The haemoglobin was 9.5 Gm. per 100 ml., white blood cell count was 10,250 per cu.mm. with a differential count of 28% segmented, 1% bands, 4% eosinophils, 65% lymphocytes and 2% unidentified cells. The child had six episodes of respiratory infection, bilateral pneumonia, anaemia and skin infections. The liver was soft and palpable, but the spleen and lymph nodes could not be felt. Clinical diagnosis: possible aplastic anaemia, lymphocytosis and granulocytopenia.

CASE 17: The patient was a thirty-two year old female with a short history of fatigue and shortness of breath. Laboratory data revealed a haemoglobin of 8.2 Gm. per 100 ml., white blood cell count of 3,000 to 5,000 per cu.mm., with neutrophils never exceeding 15%. Bone marrow aspiration showed slight hyperplasia of all cell lines. Two years previous to admission the patient had received a short course of Pyramidon for headaches. She received five blood transfusions. Clinical diagnosis: idiopathic neutropenia and iron deficiency anaemia.