FETOMATERNAL LEUKOCYTE INCOMPATIBILITY [1, 2]

By ROSE PAYNE and MARY R. ROLFS

(From the Department of Medicine, Stanford University School of Medicine, San Francisco, Calif.)

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Iso-leukoagglutinins are known to develop primarily in multitransfused patients (1–6). A possibly different kind of agglutinin for white cells, such as that related to aminopyrine leukopenia, has also been described (7). The therapeutic administration of blood and of aminopyrine may not be the only stimuli for leukoagglutinin production. Two groups of investigators, Dausset, Nenna and Brecy (8) and Whyte and Yee (9), have drawn upon the analogy to Rh antibody formation, and have discussed the possibility that fetal leukocyte antigens might act as stimuli to the mother. Killman (10) considered this potential source of antigen in his investigations of leukoagglutinins in collagen diseases, but he was unable to find any correlation between pregnancies and leukoagglutinin formation. The studies presented in this paper demonstrate that fetomaternal leukocyte incompatibility may induce leukoagglutinin formation in the mother, and suggest that some leukocyte factors, as demonstrated by their corresponding leukoagglutinins, may be genetically transmitted to the offspring.

METHODS

Technique of the test for leukoagglutinins. The details of the test for leukoagglutinins were presented in previous publications (2, 3). In this study, the test leukocytes for iso-leukoagglutinins were obtained from 10 persons of red cell blood group O; these were healthy donors whose red cells differed in their Rh factors. All inactivated sera were tested against the entire panel of 10 leukocyte donors. A leukoagglutinin test was considered positive when different samples of serum obtained from the same individual produced compact clumping of the leukocytes of a panel member on two different occasions. The sera selected as positive in this series were those which produced clumping of two or more members of the leukocyte panel. Weakly reacting sera were occasionally observed. These induced very weak clumping, or only agglutinated one member of the leukocyte panel. They were arbitrarily not included among the positive sera. Some of the weak reactions could be attributed to the adherence of leukocytes to noncellular material in serum; others seemed to be related to the gradual deterioration of the test leukocytes with time. In order to minimize these sources of error, fresh leukocytes were obtained daily. Different members of the leukocyte panel reacted with a single test serum so as to give titer differences no greater than those inherent in the serial dilution method of titration. The patients' own leukocytes were employed in tests for autoleukoagglutinins. When red cell antibodies other than anti-A or anti-B were present in test sera the test leukocytes were selected so that the accompanying red blood cells would not contain the conflicting factors. In family studies, in which sera from persons of one ABO blood group were tested against leukocytes obtained from persons of another ABO blood group, the anti-A and anti-B isoagglutinins of the sera to be tested were removed by absorption with group A and/or B washed red cells. In order to prevent absorption of the leukoagglutinin in the latter process, the leukocytes were removed from the whole blood prior to washing the red cells.

Sources of sera. Sera were collected from three groups of women. Group I included 144 obstetrical clinic patients selected with the sole qualification of multiparity; all women had had four or more pregnancies. The number of pregnancies included abortions or miscarriages, still births, premature births and full term births. No distinction was made regarding the length of time of fetal development. The majority of the women were in the third trimester while a few were in the second trimester of pregnancy. It was thought that more easily detectable amounts of antibody might be present in parous women during late pregnancy. Group II consisted of eight patients who were referred to us for study because of special problems. They were included because they showed varied circumstances in which leukoagglutinins could be found in parous women. The majority of Group II had Rh antibodies. There was A-O incompatibility between newborn and mother in one (Patient No. 26), another (Patient No. 32) was referred because of an anemia of unknown origin, and the last had had a febrile transfusion reaction (Patient No. 33). Group II was not selected at random and therefore was not used in the estimation of incidence of leukoagglutinins in parous women. Group III, a control series, consisted of 20 nontransfused healthy women who had never been pregnant. These women were selected so that the age

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range corresponded to the age range of Groups I and II. All of the women in the three groups were between 18 and 45 years of age.

Most of the sera were collected in the prepartum period. This permitted the collection of frequent samples and of a considerable quantity of sera. In addition, sera were collected at the routine six weeks postpartum visit, and also at longer intervals postpartum whenever possible. Sera were stored in the frozen state.

RESULTS

Incidence

The incidence of leukoagglutinins in 144 pregnant multiparous women, Group I, and in the control group of 20 women, Group III, is presented in Table I. Leukoagglutinins were found in 25, or 17 per cent, of the 144 multiparous women. They were completely absent in the controls who had never been transfused or pregnant. The fetus appeared to be the only stimulus for leukoagglutinin production in 18, or 68 per cent, of the 25 women, since this number had never received transfusions or blood injections.

General data regarding Groups I and II, in both of which leukoagglutinins occurred, are listed in Table II. Leukoagglutinins were already present in some of the women during the second trimester; however, most of the women were seen for the first time during the third trimester. The maternal sera agglutinated a minimum of two members of the leukocyte panel and a maximum of nine members. The majority reacted with three to seven members of the leukocyte panel. The leukoagglutinin titers ranged from 1:1 to 1:128. In the main, the titers were relatively low, not exceeding 1:8, which is similar to the titers found in the sera of multi-transfused patients (2). Auto-

TABLE I

Incidence of leukoagglutinins in pregnant, multiparous women

Subjects	No. tested	No. with LA*
Group I		
Pregnant multiparous women	144	25
With transfusions	36	7
Without transfusions	108	18
Group III		
Control women, never transfused or pregnant	20	0

* Leukoagglutinins.

TABLE II

General data on multiparous women with leukoagglutinins

Patient number	Number of Pregnancies	Number of Transfusions	Antibodies to WBC*	Antibodies to RBC†	Remarks
			Group I		
1	5	0	1:8‡	−§	
2	7	0	1:8	−	
3	6	0	+‖		
4	5	0	1:1	−	
5	7	5	1:4	−	
6	7	2	1:4	−	Febrile reaction on first transfusion
7	9	0	1:4	−	
8	9	1	1:2	−	
9	8	0	1:2	−	
10	5	1	1:4	−	
11	6	0	1:8	−	Sickling family
12	4	0	+	−	
13	7	0	+	+	Anti-Le^a
14	8	3	1:2		
15	6	0	1:8	−	
16	7	0	1:2	−	
17	5	0	1:1	−	
18	11	0	1:2	−	
19	6	0	+	−	
20	6	0	1:2	−	
21	5	0	1:2	−	
22	9	0	1:4	−	
23	5	0	1:128	−	
24	4	3	1:8	−	
25	8	11	+	−	
			Group II		
26	6	6	1:128	+	Immune anti-A
27	3	0	+	+	Anti-D 1:2,048
28	7	9	1:2	+	Anti-C 1:2 Anti-D 1:32, febrile reaction on second group of transfusions
29	7	0	1:2	+	
30	6	6	+	+	Anti-D 1:32
31	3	0	+	+	Anti-D 1:16
32	4	1	+	−	
33	6	5	1:1	−	Febrile reaction on second transfusion

* White blood cells.
† Red blood cells.
‡ Titer.
§ Negative agglutination test.
‖ Positive agglutination test, not titered.

leukoagglutinins were not present in the 10 women so tested.

Leukoagglutinins developed in women of red cell groups A, B and O. None were found in the five women of group AB who were examined. Anti-red cell antibodies occurred simultaneously with antibodies for white cells in some of the sera. Sufficient data were not obtained to determine whether or not Rh sensitized women produced leukoagglutinins more readily than other women.

Role of transfusions

The relation of transfusions to leukoagglutinin production in women of Groups I and II is shown in Table III. The majority of the transfusions were administered to these patients when they were hospitalized in other institutions and prior to this study. In all but 4 of 41 patients, the transfusions were for blood loss arising in prior postabortal or puerperal states. None of the women suffered from primary blood disorders or diseases of the collagen system. With two exceptions no patient had received more than six transfusions. Two patients with leukoagglutinins, Nos. 25 and 28, had received a total number commensurate with the number required for leukoagglutinin production in some individuals (2–6, 11). While the total number of units which they received was large, in practice several units were given at the same time. Therefore, the number of stimulations may have been insufficient for leukoagglutinin production. Patient No. 25 is of interest because upon the first testing late in the eighth pregnancy she did not possess a leukoagglutinin. She had received a total of three transfusions following postpartum hemorrhages in 1945 and 1951, the last of which was six years before the tests for a leukoagglutinin. A week after the test, during delivery of a still-born infant, uterine rupture occurred. With the attendant surgery the patient received eight more units of blood without reaction. Two weeks later, a leukoagglutinin became demonstrable for leukocytes that were not agglutinable earlier. If it had been possible to test the leukocytes of the newborn and the father, the origin of the leukoagglutinin might have been specifically attributed to either the transfusions or the fetus. Patient No. 28 received transfusions on three occasions following abortions and during a hysterectomy. In our earlier experience with 25 hematologic patients (men, women and children), who were followed during transfusion therapy, eight or more units of blood administered at intervals of days or weeks were usually necessary for leukoagglutinin production. In 61 patients (children and men exclusively) examined for leukoagglutinins but not followed throughout transfusion therapy, the median number of transfusions received prior to detection of a leukoagglutinin was 15 units.

TABLE III

Number of transfusions received by 152 multiparous women

No. of transfusions	No. of women	
	With LA*	Without LA
0	21	90
1	3	11
2	1	10
3	2	6
4		2
5	2	
6	2	
7		
8		
9	1	
10		
11	1	
Total	33	119

* Leukoagglutinins.

Febrile transfusion reactions were noted only in the histories of three of the women with leukoagglutinins, Nos. 6, 28 and 33 (Table II). The transfusions producing the febrile reactions were not given during the current pregnancy. In two of the women, Nos. 6 and 33, only leukoagglutinins were found; Patient No. 28 had a red cell agglutinin as well. Patient No. 6, who was found to have a leukoagglutinin during her sixth pregnancy, developed a febrile reaction following her first transfusion given after her third pregnancy. Patient No. 28 developed a reaction following her second group of transfusions after her seventh pregnancy. Patient No. 33, after her second pregnancy, reacted with chills and fever to four out of five units of blood following the second series of transfusions. This occurred during cesarean section. "Leukocyte poor" blood was given to this patient without reaction at the time of section for the current pregnancy. Evidence for febrile transfusion reactions was not found in the histories of women who were transfused but did not possess demonstrable leukoagglutinins. It would appear that very few transfusions might induce febrile reactions in women previously stimulated by fetal leukocyte antigens.

Stimulus for leukoagglutinin formation

In the foregoing material, it was demonstrated that multiparous pregnant women may have leukoagglutinins in the absence of transfusions or intramuscular blood injections. In order to provide further evidence that the leukoagglutinins in multiparous women, both transfused and non-

transfused, were probably the result of fetal stimulation, the maternal sera, in as many instances as possible, were tested against the leukocytes of the newborn infant and the spouse. If these leukoagglutinins were due to the fetal stimulation postulated, then the maternal sera should agglutinate the leukocytes of some of their respective newborn infants. If the inheritance of the leukocyte factors was analagous to that of the red cell groups, then it would be anticipated that the leukocytes of *all* fathers (spouses) would be agglutinated by the maternal sera containing the leukoagglutinins. In other words, some of the infants could be expected to have inherited a paternal leukocyte factor absent in the mother. The agglutination reactions in Table IV represent the results of 14 family studies in which the maternal serum was tested against either the leukocytes of the newborn, the spouse, or both. The finding that 10 of 12 newborns tested gave positive agglutination tests was consistent with the proposed hypothesis. Examination of the other siblings in Family 2 (Figure 1), in which the newborn's tests were negative, indicated that three other children appeared to have inherited the father's leukocyte antigen. Their leukocytes could have constituted the stimuli which induced the maternal agglutinin. Thus negative results with a current newborn do not invalidate the evidence for fetal stimulation. This patient's last pregnancy, in which leukocyte stimulation could have occurred, was five years before the test sample was drawn. It is plausible that the agglutinin or the effects of stimulation from an earlier pregnancy would still be present in this mother. In this laboratory leukoagglutinins have been found in a male patient five years after stimulation from blood transfusions. They were also found in six mothers six to nine months, and in two mothers a year, after delivery.

When the spouses' leukocytes were tested with the maternal sera, all but one (12 out of 13) gave positive agglutination. In Family 6, in which the leukocytes of the newborn were agglutinated and the leukocytes of the alleged father were not, either the hypothesis of direct inheritance from father may be incorrect or the man tested may not have been the true father. On the basis of erythrocyte factors, the spouse could not be excluded as the *de facto* father. The mother had had children with more than one spouse. It must also be recognized that this woman had received two units of blood on a single occasion 13 years before.

TABLE IV

Reaction of maternal leukoagglutinins with leukocytes of their newborn infants and their spouses

Sera of mothers	No. of transfusions	Leukoagglutination test WBC* of Father	Leukoagglutination test WBC* of Newborn
1	0	++++†	++++
2	0	++++	−‡
3	0	++++	++
4	0	NA§	+++
5	5	+++	+++
6	2	−	++++
7	0	++++	++++
8	1	+++	−
9	0	++++	++++
10	1	++++	++++
11	0	++++	+++
12	0	++++	++++
32	1	++++	NA
33	5	++++	NA

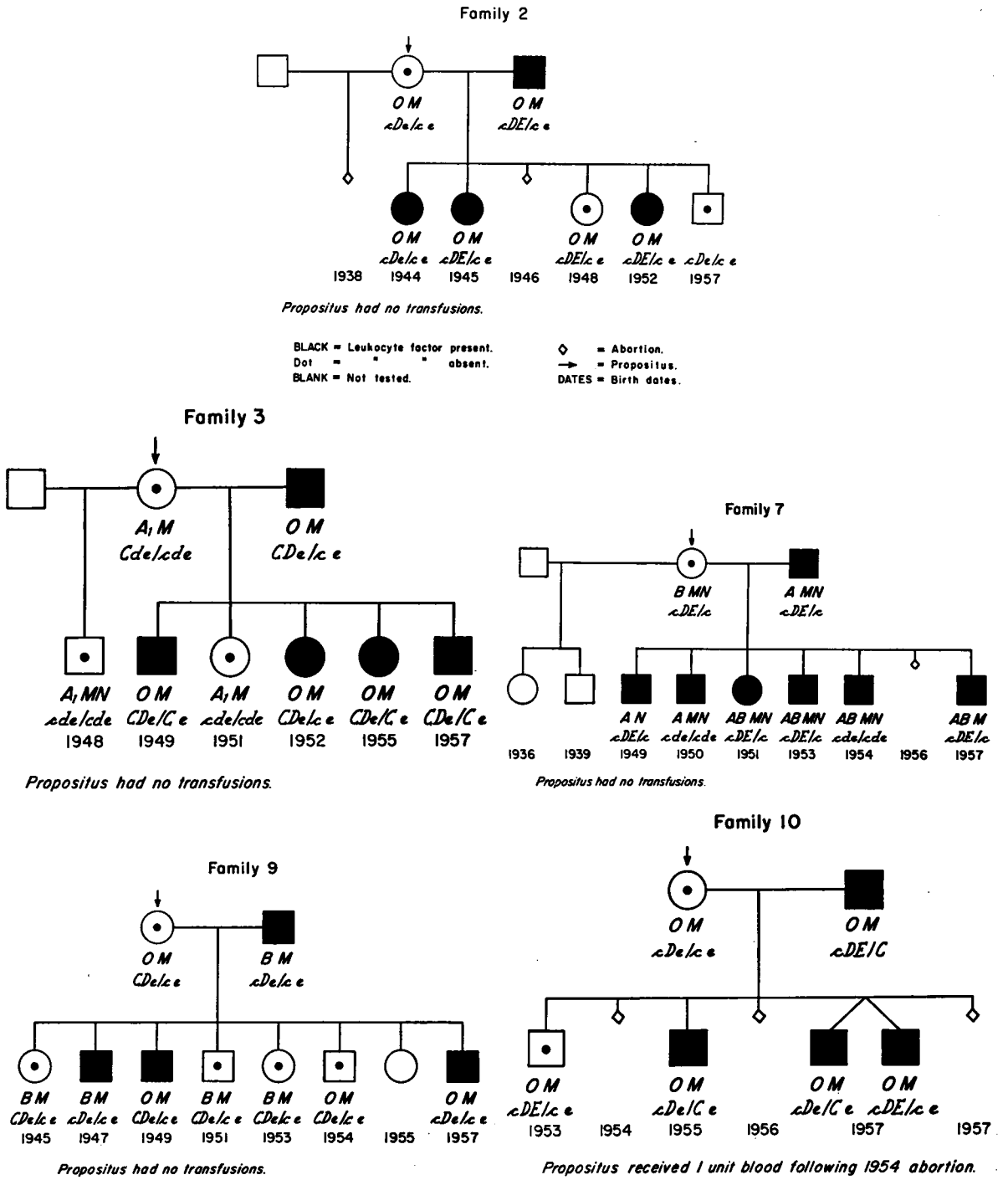
* White blood cells.
† ++++, +++, ++ indicate decreasing degrees of agglutination.
‡ Negative agglutination test.
§ Not available.

There were 11 families in which both father and infant were tested at the same time. Among these, eight pairs gave positive agglutination tests for both members; the infants in two families and the father in one gave negative agglutination tests. In six of the eight families with positive tests for both newborn and spouse, there was no history of stimulation by transfusion or blood injection. In these six families stimulation appears to have come from the fetus alone.

Family studies

More extensive family studies to demonstrate the manner in which the leukocyte factors are inherited have just been initiated. The occurrence of the leukocyte factor in each of five representative families (Families 2, 3, 7, 9 and 10) is illustrated in Figures 1 through 5. A leukocyte factor or combination of leukocyte factors appear to have been transmitted from the father to some but not all of the children in the families studied. In Family 7 all of the children appear to have inherited the factor found in the father. In this limited series of family studies there did not appear to be a relation between ABO and Rh inheritance on the one hand and leukocyte factor inheritance on the other. In order to consider the question of

dominance with regard to leukocyte antigen inheritance, the leukocytes of Family G were examined (Figure 6). The mother of Family G did not possess leukoagglutinins. This family was selected because both mother and father possessed a leukocyte factor which reacted with the maternal leukoagglutinin from Family 23. The possibility existed that one of the children would not possess



FIGS. 1–5. FIVE FAMILY STUDIES DEMONSTRATING THE DISTRIBUTION OF DIFFERENT LEUKOCYTE FACTORS AS SHOWN BY THE RESPECTIVE MATERNAL SERA

the leukocyte factor. The question of dominance of this leukocyte factor was not answered since all five children possessed the leukocyte factor. This family illustrated the presence of a leukocyte factor in three generations. In examining these pedigrees it must be kept in mind that the leukocyte factors detected are not the same in the different families. Each of the sera employed reacted differently with our panel of 10 leukocyte donors so that no two sera were identical. There was some overlapping in behavior of the agglutinins with respect to some donor leukocytes, suggesting that these sera contained antibody for more than one leukocyte factor.

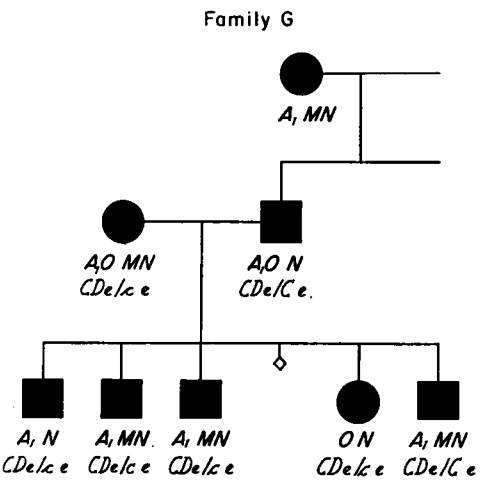


FIG. 6. FAMILY STUDY WITH SERUM OF PATIENT NO. 23 ILLUSTRATING THE PRESENCE OF A LEUKOCYTE FACTOR IN THREE GENERATIONS

DISCUSSION

The primary assumption in these studies is that fetomaternal leukocyte incompatibility may exist, and therefore leukoagglutinins ought to occur in a certain number of women as the result of fetal stimulation. That antigenic differences occur in human leukocytes is known, for these have been demonstrated in *in vivo* and *in vitro* studies related to febrile transfusion reactions. However, these differences have not yet led to the separation of clear-cut leukocyte types. It was reasoned that since erythrocytes may pass from fetus to mother in humans (12–14), leukocytes or their antigens might also cross from fetus to mother.

There was some suggestive evidence of fetomaternal leukocyte incompatibility in our earlier work on the incidence of leukoagglutinins in hematologic patients. An anemic, pregnant woman who had never received blood by injection or transfusion was found to have a leukoagglutinin (Patient No. 32 in this series, No. 321 in the original series) (2). Additional evidence was accumulated in studies on febrile transfusion reactions in men and women with leukoagglutinins (15). It was noted that women developed transfusion reactions with fewer units of blood than did men; 52 per cent of 25 female patients developed a transfusion reaction with five or fewer transfusions, as compared to 10 per cent of 31 male patients. All of the 13 women with five or fewer transfusions had been pregnant.

The present studies demonstrate the existence of fetomaternal leukocyte incompatibility, as shown by the production of leukoagglutinins in maternal sera which were capable of agglutinating the leukocytes of some of the children and their fathers. The findings of leukoagglutinins in 21 multiparous pregnant women who had never received intramuscular blood injections or transfusions suggested that a fairly large number of individuals were stimulated by the fetus. It will be interesting, as our information increases, to compare the number of mothers with leukoagglutinins to the number of Rh negative women who become sensitized by an Rh positive fetus. Rh positive fetuses in Rh negative women result from two kinds of marriages, Rh negative women with homozygous and with heterozygous Rh positive men. Thus about one-tenth of all pregnancies in Caucasian populations could theoretically result in incompatible mother-fetus interaction. The incidence of Rh disease in the newborn is, of course, much lower than the incidence of genetically unfavorable pregnancies. Clemens and Walsh (16) in a study of 5,694 pregnancies in Rh negative women showed that the per cent of Rh negative women with Rh antibody increased with successive pregnancies. They found 6.6 per cent with Rh antibody in the second pregnancy, 11.6 per cent in the third pregnancy, 17.6 per cent in the fourth pregnancy, 25.4 per cent in the fifth pregnancy and 27.5 per cent in those with six or more pregnancies. Insofar as incompatibility for leukocytes is concerned, data have been presented here that 18, or 13 per cent, of 144 pregnant parous women unselected except for multiparity possessed leuko-

agglutinins as the result of fetal stimulation alone. One-fourth of the women with leukoagglutinins in Group I had received transfusions. In all but one, there were few transfusions relative to the number required for leukoagglutinin production either in women who had never been pregnant, or in men. Therefore, probably all 24 women, or 17 per cent, had developed leukoagglutinins from fetal stimulation. These figures of incidence of leukocyte-incompatible marriages are not the minimum since the patients concerned had had numerous pregnancies. However, since all the leukocyte types are not yet known this number may not be the maximum. If a large series with fewer pregnancies were examined, the proportion with leukoagglutinins might be lower than that presented here of 13 to 17 per cent. In the course of this investigation, women of Group II with only three pregnancies were found to have leukoagglutinins in their sera.[3] Whether there are leukocyte antigens capable of stimulating demonstrable leukoagglutinins during the first pregnancy is not yet known.

Until this time, the main clinical significance of leukoagglutinins has been their responsibility for many instances of the common complication of transfusion therapy, the febrile transfusion reaction (3). The demonstration that women may possess leukoagglutinins following fetal stimulation when leukocyte incompatibility exists, may explain, in part at least, why some women have had febrile transfusion reactions with a history of no or few transfusions. Since these transfusion reactions due to leukoagglutinins can be prevented by the administration of blood from which the majority of donor leukocytes has been removed (3, 17, 18), precautions should be taken to prevent this type of febrile transfusion reaction in parous women. Emphasis should be placed on the fact that parous women may develop transfusion reactions with far fewer transfusions than do men. In this hospital, reactions of the kind associated with leukoagglutinin activity have been observed in several women upon the first transfusion. One must not assume that the leukoagglutinin found during pregnancy disappears immediately and will not therefore induce reactions at some later date. In all of the mothers who were tested six weeks postpartum the leukoagglutinins were present; those tested at two, five, seven and nine months and one year postpartum still had demonstrable leukoagglutinins. A transfusion reaction associated with leukoaglutinins was observed in a woman following her first transfusion given more than 15 years after her last pregnancy. This reaction could not be attributed to any other agent; moreover, it was not possible to check her husband or her children to establish the origin of her leukoagglutinin. There is a tendency to minimize the effect in the patient of the nonhemolytic febrile transfusion reaction. Many of these reactions on the surface seem mild. However, the mechanism of the reaction associated with leukoagglutinins has not been clarified nor have possible residual effects been assessed. Until the effects are better understood, it might be well to keep in mind some of the severe complications seen in the critically ill patients described by Brittingham and Chaplin (17). That febrile transfusion reactions will occur in women with leukoagglutinins seems reasonably clear, but the meaning of leukoagglutinins in the maternal sera for the fetus or the newborn is not clear. Investigation to date has not shown a consistent neonatal effect. A transitory leukopenia has been found infrequently. Detailed results on the effect, if any, upon the newborn will be presented when more information has been accumulated.

A most interesting possibility arises from the finding of leukoagglutinins in nontransfused women. This particular kind of antileukocyte serum may provide the tool necessary for the separation of the different human leukocyte antigens. The search for distinct and separate leukocyte types has been attempted with limited success (19, 20). The lack of clear-cut results in the past may have been due to the fact that sera from persons with multiple transfusions were employed. The use of leukoagglutinins from maternal sera should provide better results, for the antibodies will have been derived from a more limited antigenic stimulus, *i.e.*, the husband's leukocyte factors as they are inherited by the children. Preliminary studies of these maternal sera indicate that some react in a

[3] Three nontransfused women in their second pregnancy have since been found to have a leukoagglutinin which agglutinated the leukocytes of their newborn. Two of the women were apparently stimulated by the first pregnancy. The third has not been tested in this respect.

similar manner with a panel of leukocytes. At this time, a leukoagglutinin analagous to anti-D (anti-Rh_0) in its incidence has not been detected. A marked advantage would arise from the differentiation of types of leukocyte antigens. If the leukocytes could be typed, then febrile transfusion reactions might be prevented by proper matching of leukocytes rather than by removal of the leukocytes from donor blood. This latter method is more wasteful of time, equipment and blood than a typing procedure would be.

It cannot be clearly stated whether or not the leukocyte factors detected by leukoagglutinins from parous women are inherited as simple dominants. They were present in seven families for two successive generations and in one family for three generations. The limited family evidence is compatible with the assumption that the leukocyte factors are inherited either as dominants or recessives. Because of the need for additional normal physiological characters in the study of inheritance in man, the leukocyte factors, which may well be inherited in a relatively simple manner, may prove to be a valuable supplement. Studies concerning the inheritance of the leukocyte factors are being continued.

SUMMARY

Data on the occurrence and nature of fetomaternal leukocyte incompatibility have been presented.

1. Leukoagglutinins were found in 25, or 17 per cent, of 144 pregnant multiparous women. The fetus appeared to be the only stimulus for leukoagglutinin formation in 18, or 68 per cent, of the 25 women since these had never received transfusions or intramuscular injections of blood.

2. Iso-leukoagglutinins of the maternal sera agglutinated the leukocytes of 10 of 12 of their respective newborn infants, and 12 of 13 of their respective spouses, thus suggesting the inheritance by the offspring of the father's leukocyte factor. Maternal sensitization to leukocytes appears to develop in a manner analagous to Rh sensitization.

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