The Development and Persistence of Leukoagglutinins in Parous Women

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Leukoagglutinins are known to develop in pregnant women in the absence of previous transfusions or blood injections. The provoking antigens appear to reside in the tissue of the fetus, presumably the leukocytes. The experimental data which supports this concept are the findings that the sera of certain gravid women agglutinate the leukocytes of their respective husbands and some of their offspring. The production of leukoagglutinins in pregnant women seems in this respect to be analogous to that for Rh antibodies. Clinically, leukoagglutinins originating from the antigenic stimulus of the fetus are frequently responsible for febrile reactions following the very first transfusion of parous women. These observations constitute the main features of current knowledge regarding leukoagglutinins of the nontransfused woman. The detailed circumstances surrounding the development of agglutinins for leukocytes in the pregnant woman have not been defined. The number of pregnancies required for the production of demonstrable antibody and the incidence of sensitization relative to increasing parity are not known. How early in gestation leukoagglutinins occur, how long after delivery they persist, and the specific leukocyte antigens which initiate their formation remain to be explored. The studies presented here deal with the character and natural history of leukoagglutinins produced in response to the stimulus of incompatible fetal tissue.

Methods

The precise details of the method for detection of leukoagglutinins have been described in earlier publications. Sera were obtained (1) from a control group of 50 nulliparous women who had neither been transfused nor received blood injections, and (2) from 406 women with varying parity, making a total of 456 women. In the latter group, 357 were currently gravid while the remaining 49 were primiparous women who had recently delivered. Except where specifically stated, primiparous and gravid women had not been transfused. The first sample of serum from a pregnant woman was usually taken during the second or third trimester.
When leukoagglutinins were demonstrable, serial samples of serum were gathered both pre- and postpartum. These were stored in the frozen state.

The test leukocytes were obtained (a) from the husband and/or the children of each woman being studied, and (b) from a leukocyte panel of ten donors of red cell blood group O. The husband’s leukocytes were selected as the screening cell in determining the relationship of parity to the incidence of leukoagglutinins. His leukocytes were also used in assessing the titers of maternal sera. To ensure comparable titers, all the serial serum samples of the patient were tested simultaneously with a single sample of his cells. The leukocytes of the offspring were utilized in family studies to identify the potential sources of leukocyte antigen which could have stimulated the mother to produce leukoagglutinins. The panel of leukocytes was employed to compare the agglutination reactions of the serum of one woman with that of another and to compare the specificity of the pre- and postpartum serial samples of sera from a particular woman. Although the panel was arbitrarily selected without knowing the leukocyte antigen composition of the individual donors, in practice these cells appeared to include almost all the antigens that the sera under investigation could identify. Only three of the sera from 68 women with leukoagglutinins failed to agglutinate any of the panel members. These sera agglutinated the leukocytes of family members.

RESULTS

Incidence of Leukoagglutinins

Leukoagglutinins were present in the sera of 74 (18 per cent) of 406 gravid women. Primarily two groups, American Negro and Caucasian women, were studied. White cell agglutinins were found in 34 of 230 (14.8 per cent) gravid American Negro women and 29 of 185 (15.7 per cent) gravid American women of Caucasian origin. The sera from a small number of Asians—Indian, Chinese, Filipino and Japanese women—contained leukoagglutinins but the group size was insufficient to allow incidence comparisons.

Relationship of Parity to Leukoagglutinin Formation

In an earlier report from this laboratory on leukoagglutinins in pregnant women,¹ those with five or more pregnancies had been selected. This approach had been utilized to establish the occurrence of leukoagglutinins in the nontransfused pregnant woman, but gave no information on the number of pregnancies required to produce sensitization. Current work was directed to determining the relationship of the number of previous pregnancies to the incidence of this agglutinin in currently gravid women. Several conclusions may be drawn from the results of leukoagglutinin tests on the women of the present study (table 1). The enlargement of the control group beyond that in the initial study¹ strengthens the observation that leukoagglutinins are not found in the absence of pregnancy in the healthy nontransfused individual. It should be especially noted that women during their first pregnancies do not possess demonstrable leukoagglutinins. Because none of the first 35 primigravidous women studied had leukoagglutinins present in their sera during gestation, an additional group of 49 women were examined following their initial delivery. If the antigenic stimulus reached the mother late in pregnancy or only at parturition, it was thought that isoagglutinins might be found postpartum. The sera of 41 women were obtained two to four days after parturition and sera from the remaining eight were collected six weeks postpartum. Only one female
Leukoagglutinins in Parous Women

Table 1.—Relationship of Parity to Occurrence of Leukoagglutinins in 456 Women

<table>
<thead>
<tr>
<th>Number of the current pregnancy</th>
<th>Number of patients investigated</th>
<th>Number of patients with leukoagglutinins</th>
<th>Percentage of patients immunized</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>86*</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>5 or more</td>
<td>177</td>
<td>43</td>
<td>24</td>
</tr>
</tbody>
</table>

*Forty-nine of these were examined for the first time after delivery. The single case with leukoagglutinins was demonstrated postpartum.

In the postpartum group possessed leukoagglutinins. This subject was first tested six weeks after delivery. There was no doubt that her serum contained a leukoagglutinin as her serum agglutinated both the leukocytes of the newborn and the newborn's father. Subsequent serial samples obtained from this subject also agglutinated the leukocytes of panel members. As with the Rh antigens, two pregnancies were required for leukoagglutinin production in most instances; 13 of 68 (19 per cent) of the women possessed a leukoagglutinin during the second pregnancy. There was not a significant increase in the percentage of women immunized during succeeding pregnancies.

Although the primary objective was to study nontransfused pregnant women, three primigravidous women with a single previous transfusion were unintentionally included. Two of these subjects had been transfused five years prior to pregnancy and one had been transfused during the course of pregnancy. None of the three had leukoagglutinins. From these few cases, no inference can be drawn on the likelihood of sensitization by the combination of a single pregnancy and a transfusion.

Leukocyte Antigens of Family Members

The problem posed by finding that two pregnancies were usually required for the production of leukoagglutinins was: Did the second child alone, or did both children possess this potentially stimulating leukocyte antigen? To answer this question, family studies were undertaken to test if the leukocytes of the husband and the offspring from the two pregnancies would be agglutinated by the maternal serum. Four representative pedigrees of families with two offspring are illustrated in figure 1. The leukoagglutinins were all detected during the second pregnancy. These women had not been previously tested. In each instance, the leukocytes of the first and second child both contained the potentially stimulating antigen. An exception to this finding was not found. Twelve larger families were also investigated. Invariably, when a leukoagglutinin was found, at least two children possessed the provoking leukocyte antigen. Usually more than two of the children, not necessarily the first two, had the stimulating antigen. Six representative pedigrees of the larger families showing the number and order of offspring with stimulating antigens are presented in figure 2. From the family studies, it may be seen that a leukoagglutinin may be present in the mother during a gestation in which the fetus lacks the provoking antigen (see pedigree of families Str, Cur and Fre).
Development, Persistence and Specificity of Leukoagglutinins

An inquiry into the earliest time during the second pregnancy when leukoagglutinins might be detected was not specifically undertaken inasmuch as most of the women were tested during the last two months of pregnancy. However, some data on the occurrence of the antibody early in the second pregnancy were accumulated. In one subject the agglutinins were observed during the third month of gestation, in another during the fourth month, and in two others during the fifth month. Their presence early in gestation suggests either the passage of cells or antigens during the course of fetal development followed by an anamnestic response to them, and/or continuing manufacture of antibody in response to the sensitizing dose received at parturition of the first child.

Additional investigations into the development of leukoagglutinins during gestation and the persistence of antibody formation after delivery were conducted in the following manner. Samples of serum were obtained from all of the women possessing leukoagglutinins on their routine visits to the prenatal clinic, at the six weeks postpartum examination and thereafter at approximately six month intervals. The total postpartum period over which 31 mothers were repeatedly tested varied from six weeks to four years, depending on the persistence of agglutinins and availability of the subjects. Each sample of serum was tested against the same ten-member panel of leukocytes in order to com-
Fig. 2.—Six representative family studies showing the number and order of offspring with leukoagglutinin provoking antigen.
Table 2.—Activity of Leukocagglutinins of Four Women During Gestation and After Delivery

<table>
<thead>
<tr>
<th>Samples of serum</th>
<th>Leukocyte Panel</th>
<th>Titer with leukocytes of husband</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Prepartum 7 mo.</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>5 mo.</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>3 mo.</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>1 mo.</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>At delivery</td>
<td>-</td>
<td>...</td>
</tr>
<tr>
<td>2 mo.</td>
<td>-</td>
<td>...</td>
</tr>
<tr>
<td>Postpartum 6 wk.</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>4 mo.</td>
<td>-</td>
<td>...</td>
</tr>
<tr>
<td>7 mo.</td>
<td>-</td>
<td>...</td>
</tr>
</tbody>
</table>

I. Patient Alt.—Illustrating no change throughout entire gestation. Patient had seven pregnancies; all five living children had the provoking antigen.

Prepartum 4 mo. - 3 - 3 - 3 - 4 - 4 - 4 - 4 - 1-1024
2 mo. - 3 - 3 - 3 - 4 - 4 - 4 - 4 - 1-1024
1 mo. ... ... 3 - 3 - 3 - 4 - 3 - 3 - 1-1024

Postpartum 2 mo. ... ... 3 - 3 - 3 - 2 - 4 - 4 - 1-1024
8 mo. ... ... 3 - 3 - 3 - 2 - 4 - 4 - 1-1024
14 mo. ... ... 3 - 3 - 3 - 2 - 4 - 4 - 1-1024
16 mo. ... ... 3 - 3 - 3 - 2 - 4 - 4 - 1-1024
23 mo. ... ... 3 - 3 - 3 - 2 - 4 - 4 - 1-1024

II. Patient Dom.—Illustrating increase in reactivity of leukocagglutinin during gestation with loss after delivery. Patient had one abortion followed by two living children, both with provoking antigen.

Prepartum 3 mo. - 3 - 4 - 4 - 3 - 3 - 3 - 4 - 1-1024
2 mo. ... ... 4 - 4 - 4 - 4 - 4 - 4 - 1-1024
1 mo. ... ... 4 - 4 - 4 - 4 - 4 - 4 - 1-1024
At delivery ... ... 3 - 3 - 3 - 2 - 4 - 4 - 1-1024
Postpartum 4 mo. ... ... 4 - 4 - 3 - 2 - 4 - 4 - 1-1024
7 mo. ... ... 4 - 4 - 3 - 2 - 4 - 4 - 1-1024
12 mo. ... ... 4 - 4 - 3 - 2 - 4 - 4 - 1-1024
24 mo. ... ... 4 - 4 - 3 - 2 - 4 - 4 - 1-1024
30 mo. ... ... 4 - 4 - 3 - 2 - 4 - 4 - 1-1024

III. Patient Url.—Illustrating no change during gestation and loss beginning seven months after delivery. Patient had four children (including the current pregnancy), all with provoking antigen.

Postpartum 6 wk. - - 4 - 3 - 3 - 3 - 3 - 3 - 1-1024
3 mo. - - 3 - - 3 - 3 - 3 - 3 - 1-1024
6 mo. ... ... - - 3 - - 3 - - 3 - 1-1024
Prepartum second gestation 2 mo. - - 4 - - - 3 - - 3 - 1-1024
Postpartum 3 mo. ... ... 4 - - - - - 3 - 1-1024

IV. Patient Mob.—Illustrating (1) the loss of demonstrable activity of the leukocagglutinin after delivery of the first child and (2) reappearance of leukocagglutinin during the second pregnancy.

Prepartum second gestation 2 mo. - - 4 - - - 3 - - 3 - 1-1024
Postpartum 3 mo. ... ... 4 - - - - - - 3 - 1-1024

Legend: - = no agglutination; 4,3,2 = degrees of agglutination; ... = not tested.

pare the behavior of the agglutinating samples with that taken at delivery. The activity of pre- and postpartum sera from four women is presented in table 2. During gestation, the number of members of the leukocyte panel which were agglutinated by the samples of serum from a particular woman usually remained constant—that is, there appeared to be no extension of antibody specificity. For example, the serial serum samples from patient Alt (table 2) contained a leukocagglutinin of a specificity which remained the same from the second through the ninth month of her seventh pregnancy. Occasionally the number of the leukocyte panel which were agglutinable increased as pregnancy progressed (patient Dom, table 2). Four months prepartum the serum from
Table 3.—Loss of Leukoagglutinin Activity from the Sera of 31 Women Following Parturition

<table>
<thead>
<tr>
<th>Time from parturition</th>
<th>Complete loss</th>
<th>Partial loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>1 yr.</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>2 yrs.</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>3 yrs.</td>
<td>15</td>
<td>45</td>
</tr>
</tbody>
</table>

patient Dom agglutinated two members of the panel while two months prepurtum the serum agglutinated six members. In four other patients an extension of specificity was noted, not during gestation, but shortly after parturition. The latter finding may have been due to an inappropriate sampling date during gestation. However it might be that the patient was stimulated by a large quantity of fetal blood at parturition.

In contrast to the customary static pattern of agglutination during gestation, the leukoagglutinins of many women following delivery showed considerable change in their capacity to react with the panel. Partial loss of activity of the leukoagglutinin—that is, failure to react with as many members of the panel as previously—often preceded complete disappearance from the serum. In the patient Dom (table 2), partial loss of specificity was evident 14 months postpartum. The agglutination patterns of the samples of sera from patient Uri showed no change during gestation, but loss in specificity was seen seven months postpartum. A summary describing the loss of leukoagglutinin activity from the sera of the 31 women following parturition is shown in table 3. During the first year, seven of 31 (22 per cent) of the women showed complete loss. The incidence of complete disappearance reached 40 per cent during the second year and 45 per cent at the end of the third year. On completion of the first year, half of the patients had leukoagglutinins of the same specificity as at delivery; at the end of three years all the persisting agglutinins showed partial loss of specificity. The earliest disappearance occurred less than two months postpartum in a patient (pedigree Bos, fig. 2) in whom stimulation had apparently been frequent (four times) and as recent as the last pregnancy. This would suggest the occurrence of a weak leukocyte antigen and/or an individual who did not readily produce these antibodies. In another subject the agglutinins were present in diminished form six weeks postpartum but had completely faded out at the next test period six months from delivery (pedigree Ros, fig. 2). Here there was an interval of eight years between the provoking stimuli.

Indications that leukoagglutinins of other specificity may persist for longer than the three year follow-up period were deduced from the leukocyte antigen distribution in the offspring of two families. In the Cur family, (pedigree, fig. 2) the persistence of antibody formation for eight years and seven months is suggested by the fact that the fourth child, born in 1951, was the last stimulus. The agglutinin was first detected in 1958 during the seventh gestation and disappeared 19 months postpartum. The antibody reacted throughout this period with only one member of the leukocyte panel. Cur’s leukoagglutinin may have had wider specificity eight years earlier. Conversion in three years from broad reactivity to a similar status of reaction with a single panel member was
observed in the case of Fri (pedigree, fig. 2). The leukoagglutinin of the second family (Str) is still present in the mother’s serum almost eight years after the last potential stimulus in 1952 (pedigree, fig. 2). The initial test for leukoagglutinins was during her seventh and only subsequent pregnancy in 1957. The specificity of her leukoagglutinin has narrowed over the 34 months since the last pregnancy. These apparently persistent leukoagglutinins were observed in only two multiparas in this series.

Effect of Repeated Pregnancies on the Specificity of a Leukoagglutinin

During the course of this study, eight of the women became pregnant again. This provided an opportunity to examine the influence of an additional pregnancy on the specificity of the leukoagglutinin produced in response to a previous stimulation. An apparently weak leukoagglutinin might completely disappear in the postpartum period and reappear during the new pregnancy when the fetus was capable of stimulating antibody production. An interesting case in point was the primipara who first had a demonstrable leukoagglutinin six weeks postpartum (Mob, table 2). This antibody disappeared six months postpartum but again became demonstrable during the seventh month of her second gestation. On reappearance, the specificity appeared to be the same as after the first pregnancy. Three months after delivery of the second infant, partial loss of specificity was noted. In other women, the loss of specificity of the leukoagglutinin in the postpartum period was partial, but returned during the next pregnancy to the full spectrum of original specificity. Pregnancies followed so rapidly in other women that no loss of activity of the leukoagglutinin was observed during the entire course of the study. In no case was there an increase in specificity with another pregnancy. On the contrary, when the new fetus lacked the provoking antigen, as was apparent in two cases, there was gradual loss of antibody activity throughout the new pregnancy.

Leukoagglutinin Titers

Titers of leukoagglutinins produced during pregnancy were similar to those induced by multitransfusion and usually did not exceed 1:16. The maximum titer seen was 1:1024. This occurred during the seventh pregnancy of a patient who had not been tested for leukoagglutinins previously. The firstborn died, due to a brain tumor, at four years of age. The leukocytes of her five living children were all agglutinable by maternal serum, suggesting homozygosity of the father for the leukocyte antigen concerned (pedigree Alt, fig. 2). It was not possible to test the leukocytes of the offspring from the seventh pregnancy as this fetus was stillborn. Autopsy failed to reveal either elements of structural abnormality or cause of death. The father was Rh positive, the mother was Rh negative, but there was no evidence of rhesus sensitization. For the leukoagglutinin titers, serial samples of her serum, collected prior and subsequent to delivery, were tested simultaneously with the leukocytes of her husband. A demonstrable rise in titer from 1:128 in the second month of gestation to 1:1024 in the seventh month was noted (table 2). Four months after delivery the titer returned to 1:128. In most individuals, however, a significant rise in titer was not observed during a gestation in which the fetal cells contained the provoking antigen.
LEUKOAGGLUTININS IN PARIOS WOMEN

Table 4.—The Specificity of Representative Leukoagglutinating Sera from Gravid Women with Reference to the Number of Leukocyte Panel Members Agglutinated and the Number of Times the Agglutination Pattern was Detected in the Sera of Other Women

<table>
<thead>
<tr>
<th>Leukocyte Panel</th>
<th>Aw</th>
<th>Cr</th>
<th>McK</th>
<th>Br</th>
<th>Bw</th>
<th>Va</th>
<th>dsB</th>
<th>Mac</th>
<th>MR</th>
<th>Bj</th>
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<tbody>
<tr>
<td>Serum type</td>
<td></td>
<td></td>
<td></td>
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<td>Str</td>
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<td>Dom</td>
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<td>Fri</td>
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Specificity of Leukoagglutinating Sera

The leukoagglutinins of the pregnant women exhibited many different specificities when tested against a leukocyte panel. As may be seen in table 4, the different leukoagglutinins might agglutinate any number of the members of the leukocyte panel or none of them. Forty agglutination patterns with the panel were found among the sera from 68 women. Leukoagglutinins of 18 specificities were duplicated in the sera of other women. These included two-thirds of the women found to have leukoagglutinins. Some of the leukoagglutinin types were found in two women, others in three women and two leukoagglutinins were each observed in five different women. The frequencies of the antigens identified by the latter two sera (Fri and Str) were 62 per cent and 38 per cent of a population selected at random. The number of women in whose serum representative leukoagglutinins were found are noted in table 4. Only three of the 68 leukoagglutinins studied failed to react with the panel leukocytes. The incidence of the antigen(s) detected by these three has not yet been ascertained but each of them agglutinated the leukocytes of the husband of the propositus as well as the leukocytes of their offspring. The observation that only three leukoagglutinins were negative with the panel cells suggests that the latter contained many of the leukocyte antigens capable of stimulating leukoagglutinin formation.

DISCUSSION

One of the most interesting facets of the present study is the finding that a remarkably large proportion of women become immunized during pregnancy to fetal antigens, presumably those of the leukocytes. Leukoagglutinins were found in the sera of 74 or approximately 18 per cent of 406 gravid women (table 1). This proportion, obviously weighted in favor of multiparity, is of course larger than would be expected in unselected pregnancies. If sensitization of random gravid women is estimated from only those who had one and two pregnancies (table 1), the ensuing proportion of 9 per cent of the gravid population is still considerable. Whether this exceeds the incidence of immunization to all of the red cell antigens combined cannot be answered. To
our knowledge a systematic compilation of all red cell sensitization during pregnancy has not been published. This is in part attributable to the difficulties inherent in assessing immunization to the antigens of the ABO system. Since most of the existing statistical information on red cells pertains to the Rh antigens, primarily to the antigenic determinant D (Rh), it would be of interest to compare Rh sensitization with leukocyte sensitization. The incidence of Rh sensitization often quoted is one in 200 pregnant women provided they have neither been transfused nor received blood injections.5 Some of the data supporting this statement were derived from the following representative studies. Boorman, Daley and Dodd,6 in an investigation initiated as early as 1944, found Rh antibodies (anti-D and anti-E) in one per cent of 2,000 gravid English women. In the United States, Sachs, Kuhns and Jahn7 in 1947 reported that 96, or 0.77 per cent of 12,275 pregnant women collected at random were immunized to the Rh antigens; 10 of these occurred in Rh positive women. In another study from England, Wiener, Norris and Davidson8 found the frequency of Rh immunization (anti-D) to be 0.52 per cent among 82,000 patients. Hartmann and Brendemoen9 in Norway detected Rh antibodies (anti-D) in 0.6 per cent of 75,000 pregnant women. From the data cited above, sensitization to the leukocyte antigens during pregnancy is certainly a far more frequent event than sensitization to the Rh antigens.

How can the difference between the frequencies of immunization to leukocyte and to Rh antigens during pregnancy be explained? Undoubtedly several factors, some of greater influence than others, are involved. The number of antigens, their antigenicity, their frequency in the population, the ease with which they enter the maternal circulation, and the ability of the recipient to respond to these stimuli must all be considered. Of the 65 known red cell antigens, those in the Rh system represent a small number. It might be proposed that leukocyte immunization therefore is frequent because it stems from the activity of a greater number of leukocyte antigens. The existence of many leukocyte antigens is suggested by the variety of agglutination patterns observed with the sera from pregnant women (table 4). The actual number of leukocyte antigens is unknown, so that an evaluation of the contribution that numbers make is at present not possible. On the question of antigenicity, the Rh system is singularly antigenic. Many instances of erythroblastosis fetalis in first pregnancies have been described following a single transfusion of D positive blood. Moreover, antibodies to other members of the Rh system are often detected.

The evidence on leukocyte antigenicity is contradictory. The very common occurrence of the immunization of pregnant women to leukocytes would at first glance suggest antigenicity to be an important factor. Yet, in the absence of pregnancy, usually 10 or more separate transfusions from different donors are necessary before leukoagglutinins become demonstrable.4,10 When the source of the blood has been a single donor, Brittingham11 first noted the development of leukoagglutinins after five 100 ml. transfusions administered at intervals of a week. Marchal et al.12 in a similar experiment, stimulated the production of leukoagglutinins in four recipients after seven weeks of injections. In contrast, Walford, Anderson and Doyle13 reported that leukoagglutinins may develop after the single massive exchange of blood administered during cardiac sur-
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Perhaps these latter workers encountered a particularly antigenic leukocyte type. In our laboratory, leukoagglutinins were not found in any of 15 patients 10 days to three weeks after cardiac surgery. Until leukocyte blood groups are clearly distinguished, adequate comparisons of antigenicity between these and red cell antigens cannot be made.

It may be that the key to the disparity between leukocyte and Rh sensitization will be found in the distribution of the different antigens in the population. In the Rh system, approximately 15 per cent of Caucasian pregnant women risk immunization to the most potent of the antigenic determinants D (RhD). The number of Rh negative women in the population obviously limits the group at risk. Applying these terms to leukocyte sensitization, it would seem that the lack of specific leukocyte antigens may be a common phenomenon. In other words the number of women potentially available for immunization to leukocyte antigens may constitute a greater proportion of the female population than the group subject to Rh immunization. Theoretically the optimal frequency for immunization for a blood group antigen would be 50 per cent. Using maternal sera, Payne and Hackel\textsuperscript{14} reported frequencies of the order of 20–40 per cent for eight leukocyte antigens. The range of frequency of nine leukocyte antigens investigated by van Rood, van Leeuwen and Eernisse\textsuperscript{3} was 14–85 per cent with the majority falling between 34 and 75 per cent. The distribution of these specific leukocyte antigens in the populations examined is consistent with the frequency of sensitization observed.

On the matter of ease of transmission from the fetus to the mother, fetal leukocytes have not yet been regularly identified in the maternal circulation. Since the leukocytes’ principal habitat is outside the circulation, and their location influenced in part by motility, migration of leukocytes across placental barriers would seem probable. If cellular entry is gained, leukocytes may have a selective advantage over some red cells in that natural isoagglutinins for them do not exist. The isoagglutinins anti-A and anti-B seem to play a part in eliminating incompatible Group A and B Rh positive erythrocytes from the maternal circulation and hence limit the incidence of Rh sensitization. Levine\textsuperscript{15} and others have shown that Rh hemolytic disease of the newborn is less frequent in ABO incompatible matings. While selection of leukocyte antigen types is apparently not achieved in this manner, rapid elimination of entering cells may be effected by other mechanisms. Whatever the explanation for the frequency of sensitization of gravid women to the leukocyte antigens may eventually prove to be, even more interesting will be its full biologic import, a problem for future investigations to unravel.

So far, dissimilarity to Rh immunization has been emphasized, but the results also illustrate several similarities. Two pregnancies, in each of which the fetus must contain the provoking antigen, seem to be required before either Rh or leukocyte agglutinins are readily demonstrable in the mother (table 1). This is in keeping with the absence of the so-called natural isoagglutinins and in this respect differs from ABO immunization which does occur in first pregnancies. While two pregnancies were essential in the majority of women examined, the finding of only one sensitized primiparous woman with leukoagglutinins may not reflect the true situation in this category. This mother was tested for the first time six weeks after delivery, a time which may have been
fortuitous for the detection of leukoagglutinins in this group. Unfortunately, 41 of the 49 primiparas were tested two to four days after delivery; the remaining few were examined for leukoagglutinins at the later date. A new series tested just prior to parturition and again three to six weeks later would clarify the situation following a single pregnancy. There is some precedent for expectation of further cases of primiparous women with leukoagglutinins from the follow-up studies of Wiener and Hallum on rhesus-negative primigravidae. They were of the opinion that the primary immunizing dose of Rh antigen might be received at parturition and, therefore, although Rh antibodies are not found during gestation of the first fetus, they might be found in exceptionally good reactors at some period after delivery without a further stimulus being applied. In their series of 62 women with a single pregnancy, two developed Rh antibodies postnatally, without additional immunization.

Direct evidence for the persistence of leukoagglutinins for long periods after delivery is clearly shown in the work presented here. Of the 31 women in the follow-up group, 55 per cent still had leukoagglutinins three years after parturition. The family studies indicated that some leukoagglutinins could be demonstrated in women at least eight years after receipt of the provoking stimulus. These findings relate to our previously published observations of leukoagglutinin transfusion reactions in parous women upon receipt of their first unit of blood. The time lapse between the transfusion reaction and the last pregnancy varied between two and 39 years. The continued presence of leukoagglutinins long after the provoking pregnancy is comparable to that for the Rh antibodies. In the transfusion of parous women, particularly during the child-bearing years, the significance of a high incidence of sensitization coupled with the persistence of leukoagglutinin production should not be overlooked. In our experience, approximately two-thirds of all leukoagglutinin transfusion reactions occurred in women; the majority were from the obstetric and gynecologic service. The routine crossmatch provides no assistance in the prevention of leukoagglutinin transfusion reactions for it does not detect leukocyte incompatibility. In current practice, preventive measures are undertaken primarily for the hematologic patient requiring multiple transfusions. The serum is examined for leukoagglutinins after the febrile reactions become troublesome. Subsequent prevention in these patients is accomplished by the administration of leukocyte-depleted blood. On an obstetric and gynecologic service, recurrent transfusion therapy is not customary. Usually the leukoagglutinin transfusion reaction occurs in a woman during one of the first few transfusions. For this group, an entirely different approach toward prevention might be considered. Pregnant women are routinely screened for Rh type and the presence of Rh antibodies. It would be of interest to explore the possibility of screening for leukoagglutinins the parous women on an obstetric and gynecologic service. This could most easily be accomplished by testing the woman's serum against the leukocytes of her husband. Leukocyte-poor blood could then be made available for those with antibody in their serum. When emergency did not allow time for preparation of leukocyte-poor blood, the administration of only the sedimented red cell portion of a unit of whole blood would probably reduce the incidence of febrile reactions.

The variety of specificities found in the leukoagglutinating sera from preg-
nant women has rendered the delineation of leukocyte types difficult. It seemed that maternal sera, having a more limited antigenic stimulus than the sera from multitransfused patients, might readily define the leukocyte blood groups. While the duplication in women of 18 leukoagglutinin types provides encouragement, the differentiation of the leukocyte antigen types remains a complex problem. When a given leukoagglutinin has its widest spectrum of activity at or around parturition (table 2), the serum often appears to contain two or more white cell agglutinins. Preliminary studies have shown that these can be separated by absorption. Our demonstration of leukoagglutinins of more than one specificity in a maternal serum is in agreement with the work of van Rood et al. The narrowed specificity of a serum which develops a year or two after delivery may represent a serum specific for a single antigen. Future work, for example with the leukoagglutinin from Uri (table 2) obtained 30 months post-partum, may after careful absorption be shown to be directed against a single antigen. If true, such sera would provide ready made reagents.

**Summary and Conclusions**

Leukoagglutinins were shown to occur in sera from nontransfused gravid Negro, Caucasian and Asian women. Investigations into the relationship of parity to the incidence of leukoagglutinins indicated that two pregnancies were usually required before leukoagglutinins became demonstrable in the gravid women. In their second pregnancies, 13 of 68 women (19 per cent) possessed leukoagglutinins. In families with two children, the leukocytes of both offspring contained the potential antigenic stimulus for leukoagglutinin production. In this series with increasing parity, the proportion of women immunized did not significantly differ. The significance of the frequent leukocyte sensitization was discussed.

Leukoagglutinins persisted in the sera of parous women for varying periods of time after parturition. The antibodies of 16 of 31 women could still be identified in their sera three years after delivery. The distribution of potentially stimulating leukocyte antigens in the offspring of two large families provided suggestive evidence that the formation of leukoagglutinins had persisted in the two women for eight years after sensitization.

The leukoagglutinins found in gravid women had numerous specificities. Eighteen of the 40 specificities (agglutination patterns) of leukoagglutinins were observed in the sera of other women. With increasing time after delivery, the specificity of leukoagglutinating sera tended to become narrower than the serum sample obtained at parturition.

**Summario in Interlingua**

Esseva monstrate que leucoagglutininas occurre in seros de gravidas negre, caucasian, e asian sin experientia de transfusion de sanguine. Investigationes con respecto al relation inter le antecedentes de graviditate e le incidentia de leucoagglutininas indicava que duo pregnantias esseva usualmente requirite ante que leucoagglutininas deveniva demonstrable in le feminas gravide. Durante lor secunde pregnancia, 13 inter 68 feminas (19 pro cento) possedeva leucoagglutininas. In familias con duo juvenes, le leucocytes de ambes contínea le stimulo antigenic potential pro le production e leucoagglutininana.
In iste serie con antecedentes de progressive numeros de pregnantias, le proportion del immunisate feminas non differeva significativamente. Es discutite le signification del frequente sensibilisation leucocytic.

Leucoagglutininhas persisteva in le serous de feminas con experientia de pregnantia durante varie periodos de tempore post le parturition. Le anticorpo in 16 ex 31 feminas eseva ancora identificable in lor seros tres annos post le parturition. Le distribution del potentialmente stimulante antigenos leucocytic in le prole de duo grande familias provideva evidentia satis forte in supporto del conclusion que le formation de leucoagglutininhas habeva persistite in le duo matres durante octo annos post lor sensibilisation.

Le leucoagglutininhas trovate in gravidas haheva numerouse specificitates. Dece-octo del 40 specificitates del leucoagglutininhas eseva observate in le seros de altere feminas. Con le passage de tempore post parturitiones, I: specificitate de seros leucoagglutinatorri tendeva a restringer se in comparison con illo trovate in seres obtenite al tempore del parturition.

REFERENCES


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The Development and Persistence of Leukoagglutinins in Parous Women

ROSE PAYNE and Millie Tripp