Familial Neonatal Neutropenia with Maternal Leukocyte Antibodies

By E. H. BRAUN, A. E. BUCKWOLD, H. E. EMSON AND A. V. RUSSELL

ISOLIMMUNE leukocyte antibodies have been reported frequently since the initial communication of Dausset and Nenna1 in 1952. A large literature has accumulated and was recently fully reviewed by Walford,2 who listed over 500 reports. Most of these have been concerned chiefly 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,2 the L.E. phenomenon,4,5 and, more controversially, in certain neutropenic states.6,7

Most frequently, isoimmunization to leukocytes has been described as the sequel of multiple transfusions.8,9,10,11 Payne and Rolfs have demonstrated the occurrence of leukocyte antibodies as a result of sensitization by fetal leukocytes.12 These authors were unable to attribute any pathological effects of such antibodies on the bone marrow or white cell count of the infants.

Neutropenia in the newborn has been sporadically reported in the past and these reports are summarized in two previous papers.13,14 In 1954, Ballowitz and Ballowitz15 reported a case of erythroblastosis fetalis (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 authors13,16 also postulated that neutropenic states in the newborn could be the result of the actions of maternal isoimmune antibodies on the fetus, but without direct evidence.

Quite recently, Lalezari et al.14 reported a family with multiple case of neonatal neutropenia. A leukoagglutinin in the maternal serum was active against the leukocytes of the father and of three available children. A case involving a single infant was reported by Rossi and Brandt.17

The purpose of this paper is to report a similar family in which a further pregnancy presented the opportunity for studies which have helped to clarify the mechanism of the neonatal neutropenia.

CASE REPORT

These data were obtained following the most recent (seventh) pregnancy of Isobel P., a 32 year old white woman of Anglo-Saxon origin. Her husband was of a similar racial extraction. Both were healthy and the mother's routine hematological data were quite unremarkable during the pregnancy. Her direct and indirect Coombs' tests [using CDc/cDE, KK, Fy(a+) cells] were negative.

The blood groups of the family are shown in table 1 and the family tree in figure 1. The first pregnancy was uneventful. The second terminated in a stillbirth of approximately
Table 1.—Blood Groups of P. Family

<table>
<thead>
<tr>
<th>Name</th>
<th>Year of birth</th>
<th>Blood group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>James P.</td>
<td>1923</td>
<td>O MS Ms P+ CDw/CDe kk Fy(a—)</td>
</tr>
<tr>
<td>Mother:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isobel P.</td>
<td>1928</td>
<td>O Ns Ns P+ CDw/eDE kk Fy(a+)</td>
</tr>
<tr>
<td>James</td>
<td>1948</td>
<td>O MS Ns P+ CDw/eDE kk Fy(a+) weak</td>
</tr>
<tr>
<td>Stillborn</td>
<td>1949</td>
<td></td>
</tr>
<tr>
<td>Margaret</td>
<td>1952</td>
<td>O Ms Na P+ CDw/eDE kk Fy(a+) weak</td>
</tr>
<tr>
<td>Dianne</td>
<td>1954 (died at 15 days)</td>
<td></td>
</tr>
<tr>
<td>Wendy</td>
<td>1955</td>
<td>O MS Ns P+ CDw/CDe kk Fy(a—)</td>
</tr>
<tr>
<td>Freddy</td>
<td>1956</td>
<td>O P+ CDw/eDE kk Fy(a+)</td>
</tr>
<tr>
<td>Douglas</td>
<td>1959</td>
<td>O Ms Ns P+ CDw/CDe kk Fy(a—)</td>
</tr>
</tbody>
</table>

six months' gestation, shortly after which Mrs. P. received a transfusion of fresh whole blood donated by her husband. The third pregnancy was unremarkable and a healthy child resulted. The fourth child, a girl, was born at term but contracted a neonatal infection with marked neutropenia and died of septicemia after 15 days of life. The fifth child, a girl, was normal. The sixth child, a boy, also had neonatal neutropenia and severe infection, but recovered after treatment with antibiotics and adrenal cortical steroids. The neutrophile count of this child rose to normal levels during treatment for the infection.

The last baby (Douglas) was expected in November, 1959. In view of the past events, maternal blood was obtained on two occasions before delivery and 6 and 12 weeks after birth. The baby was born on November 19, weighed 8 lbs., and was normal on physical examination. Blood counts obtained within two hours of birth were as follows:

- Hgb. 20.4 Gm. %
- R.B.C. 6.5 M./cu.mm.
- Platelets 104,000/cu.mm.

4 hours after birth:

- W.B.C. 17,000/cu.mm.
- Mature Polymorphs 16%
- Bands 3%
- Lymphocytes 67%
- Monocytes 11%
- Eosinophils 3%
- W.B.C. 8,000/cu.mm.
- Mature Polymorphs 20%
- Bands 2%
- Lymphocytes 59%
- Monocytes 14%
- Eosinophils 5%

The only abnormality was the low number of granulocytes. The subsequent leukocyte counts are shown in figure 2.

Following birth, a fall in the total white cell count was observed. The initial neutropenia became more marked but gradually rose without adrenal cortical steroid therapy. By April, 1960, the white cell count was as follows:

- W.B.C. 9,150/cu.mm.
- Mature Polymorphs 32%
- Bands 12%
- Lymphocytes 46%
- Monocytes 6%
- Eosinophils 4%

In the period between birth and April, 1960, the diminished number of neutrophils was apparently compensated by an increased number of monocytes. The latter diminished as the neutrophils slowly increased. Lymphocytes have remained within normal limits since birth.

Tibial bone marrow aspirations were performed on the eighth and twenty-eighth days
Fig. 1.—Family tree of Baby Douglas P.

of life. The first showed a marked reduction of all leukocytic elements (fig. 3). The second aspiration showed hyperplasia, most prominent in the myeloid and monocytic series (fig. 4). In both aspirations, marked dilution with peripheral blood made the differential count meaningless.

The child was discharged from the hospital on the third day of life at the insistence of his mother. Seven days after birth a small bleb was noticed on the face and white patches

Fig. 2.—Baby Douglas P.: Leukocyte picture. (Interrupted line represents average normal neutrophil count for this age group.)
Fig. 3.—Bone marrow, Baby Douglas P., eighth day of life; Atrophy of granulopoietic cells.

appeared in the baby's mouth. Because of the family history the boy was readmitted to hospital. Candida albicans was cultured from both areas. The child was placed in strict isolation, was treated with Mycostatin and the lesions cleared. He remained in the hospital, and apart from a brief period of fever and diarrhea, which responded to Neomycin, has progressed satisfactorily and he was discharged on January 24, 1960, aged sixty-six days, with a white blood count of 8,000 cells cu. mm., 25 per cent being neutrophils; segmented and band cells. He has since been cared for at home and his development has been normal.

MATERIALS AND METHODS

Leukocytes were obtained from the father and all surviving children, except Baby Douglas, from whom it was impossible to obtain adequate numbers of cells. Six unrelated donors (Group O, Rh + ve) were also investigated.

Blood was obtained by venipuncture using a two syringe technic. Needles were siliconed with Arquad, syringes with Drifilm 88 using a vaporizing technic.

The blood was defibrinated and sedimented using Dextran 250* (viscosity numer dl g 0.42) in a 4 per cent w/v solution with 3 per cent dextrose in 8.5 M. sodium chloride solution.

After sedimentation for 45 minutes, the concentrated leukocytes were incubated with the test serum and dilutions thereof for one hour. Agglutination was read both in wet fields using phase microscopy, and after drying and staining of the slides.18

All sera were heat inactivated by incubation at 56 C. for half an hour.

Controls, using Mrs. P.'s cells against her own serum and each donor's cells against his own serum, were consistently negative.

Tullis's method19 was followed in the test for leukolysins.

Absorption and elution studies were performed by the method of Killman.20

*Supplied by Pharmacia Uppsala, Sweden.
Fig. 4.—Bone marrow, Baby Douglas P., twenty-eighth day of life: Myeloid and monocytic hyperplasia.

RESULTS

Positive results were obtained using the father's cells, cells from the surviving previously affected child (Freddy), and with cells from three of the six unrelated donors. Titers did not differ by more than one dilution in these five positive cases. The average results are shown in figure 5, which illustrate the rise in titer during the last trimester of the pregnancy (Max. titer 1:512) and its subsequent fall. The antibodies were also present in the cord blood to a titer of 1:16.

Leukolysins were demonstrated four days before delivery. A fall in the leukocyte count from 20,300 cu./mm. to 7,150 cu./mm. occurred after one hour's incubation. The amoeboid motility of the white cells suspended in modified Hank's buffer decreased 25 per cent. The "lysis" index was thus 1,500, which by Tullis's standards is highly abnormal (normal range 0-24).

The leukocyte antibodies were absorbed from the maternal serum using donor's leukocytes (Group O, Rh+ve) and could be subsequently eluted. Following absorption, the maternal serum failed to agglutinate the cells it had previously agglutinated, while the eluate caused agglutination of leukocytes in titers up to 1:32.

Completely negative results were obtained with the leukocytes of the children that had been clinically unaffected.

DISCUSSION

The data obtained in the present study indicate sensitization of the mother by her husband's leukocytes with the formation of antileukocyte antibodies.
Succeeding pregnancies with fetuses 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 titers 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 with resulting neutropenia. There was no compensatory rise in the eosinophil series, though circulating eosinophils were present in normal numbers. Basophils were not seen. The marked rise in monocytes was probably a compensatory mechanism and these cells declined in numbers as the neutrophils increased. Lymphocytes appeared unaffected during the period of our observation.

Bone marrow aspiration on the eighth day of life revealed an almost complete lack of white cell precursors, while on the twenty-eighth day of life there was a marked hyperplasia of these elements. These findings posed some interesting and currently unanswerable questions. The effect of the passively transferred antileukocyte antibodies was very prolonged. This may have been due to their persistence in the circulation, by their fixation in the infant's tissues, or by a prolonged inability of leukocyte precursors to regenerate even
after the antibodies had disappeared. In the previously affected child there was hyperplasia of the marrow and neutrophilic leukocytosis after eight days of steroid therapy.

It is interesting to note that the antibody affected only the neutrophil leukocytes and had no effect on the erythroid, monocytic and lymphocytic series.

Leukocyte isoagglutinins have been provoked mainly by multiple transfusions,\textsuperscript{11} or by multiparity,\textsuperscript{12} In either case, they appeared to have no effects on subsequent infants. The particular interest of our case lay in the fact that sensitization from a single transfusion of fresh whole blood resulted in a mechanism which appeared to have a clinical effect on several subsequent infants.

When Mrs. P. was transfused with her husband's fresh whole blood in 1949, the leukocytes would be fresh and comparatively uninjured, and may be presumed to have been more potent antigenically than the old and damaged leukocytes of blood stored in glass containers. Thus may be explained the comparative rarity of acquired sensitization to transfused leukocytes, a situation which may not persist with the increasing use of plastic equipment in the storing and transfusion of blood.

In studying the erythrocyte types of husband and wife (table 1) it will be noted that their groups are very similar, particularly in regard to systems which are highly antigenic. The transfusion with the husband's erythrocytes would have therefore left the mother's antibody forming mechanisms free to form antibodies against weaker antigens, such as the husband's leukocytes.

The potency of the mother's leukoagglutinins was shown by the clinical condition of the affected infants and the demonstration of a high titer of agglutinins and of actual leukolysins immediately before delivery.

The occurrence of affected and unaffected children after sensitization suggested the presence of at least two antigenic types of leukocytes. The antigens did not demonstrate sex linkage, nor were they linked with the few antigens in which maternal and paternal erythrocytes differed. This selectivity also suggested that we were dealing with an iso- rather than an auto-antibody, as the latter are usually panagglutinins.

**Summary**

The history of a family with three cases of neonatal neutropenia is reported. During the mother's last pregnancy leukocyte antibody titers were found to be rising, culminating in a titer of 1:512 at delivery, when leukolysins and passive transfer of antibody to the fetus in the cord blood were also demonstrated. This pregnancy resulted in an infant with a severe degree of neutropenia during its neonatal period. The original sensitization was attributed to prior transfusion with the husband's whole fresh blood. The whole process can be compared with the sensitization produced by the Rh factors.
corpore al feto esseva demonstrate in le sanguine umbilical. Iste pregnantia resultava in un infante con sever grados de neutropenia durante le periodo neonatal. Le sensibilisation original es attribuite a tin previe transfusion de total sanguine fresc donate per le marito. Le integre processo pote esser corn-parate con le sensibilisation producite per le factores Rh.

REFERENCES

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