Hemolytic Disease of the Newborn Due to Anti-A,

By P. O. Hubinont, P. Latiers and T. Massart-Guiot

There is a definite correlation between fetal loss and ABO incompatible pregnancies according to various studies. On the contrary, discussion is still open whether ABO iso-immunization induces a condition identical in every way with hemolytic disease of the newborn due to anti-Rh. Some evidence shows that this may occasionally happen. More atypical forms were, however, described on the basis of serological findings, the mother's serum containing an immune antibody anti-A or anti-B, held to be responsible for the baby's illness or death.

Optimal Temperature of Agglutination in Saline

In collaboration with Professor Maurice Millet, one of us described in 1946 two cases with a history of successive deaths "in utero" at term, or even during labor, where the patient's serum contained an agglutinin more active at 37 C. than ice-box temperature and, for this reason, considered as immune. The same property was found in animal experiments of hetero-immunization. That the thermal optimum, at body temperature, of anti-A and anti-B is the result of an iso-immunization has been proved by observing the serum of two patients accidentally transfused with incompatible blood: one with A and the other with B blood. In both cases the specific agglutination in saline was optimal at 4 C. before transfusion and soon became optimal at body temperature. This property of immune anti-A and (or) anti-B serum was verified by several authors but not regularly enough by others to be considered as specific.

Agglutination in Protein Media and Sensitization to the Anti-Human-Globulin after Partial Neutralization with Blood Group Substances

More frequently the sera containing immune anti-A or anti-B exhibit a curious phenomenon described for the first time by Witebsky. The serum of his patient, after partial neutralization with blood group substance A still agglutinated A cells in protein media while it failed to do so in saline. There was thus a striking analogy between this particular anti-A and the anti-Rh "blocking" antibody. It was eventually shown that partially neutralized immune anti-A or anti-B sera sensitized the specific cells to the anti-human-globulin serum.

However, these two properties may be found in many instances of blood group O donors who had no obvious exposure to antigenic stimulation by the A or B factor.
Heat Resistance

Boorman and Dodd showed that heating the serum for 20 minutes at 75°C produced an effect similar to partial neutralization. Heated immune anti-A serum had lost the capacity of agglutinating but "blocked" A cells and sensitized them to the antiglobulin serum. Thus the action of heat upon immune anti-A was similar to that reported for anti-Rh agglutinating sera. In both cases the phenomenon suggests that "heat, by destroying the agglutinin, unmasks the immune blocking antibody". However, some data obtained from the study of heat denaturation show that some kind of combination between albumins and the antibody-globulin is responsible for the blocking effect. The agglutinating properties could be restored by suitable methods. So there is no destruction of the agglutinin, but a modification of the antibody molecule which alters the antigen-antibody reaction.

Aside from the serological problems of ABO iso-immunization, there are many variations in the clinical manifestations in the fetus or newborn, between repeated deaths "in utero" and a mild, self-limiting form (icterus praecox), barely distinguishable from physiological jaundice.

Recently the clinical features of the more frequent forms were reviewed by Mollison et al. The main points are the early and intense jaundice, the evanescent and very slight signs of hemolysis and the fact, observed by many authors, that the direct anti-globulin test is often negative.

Frequently the first-born is affected.

**CASE REPORT**

**Clinical Data**

At the 40th week of an uneventful pregnancy, Mrs. Van Im gave birth to a living female child. She had no history of abortion, had never received a blood transfusion and this was her first pregnancy.

A few hours after birth the baby became jaundiced. The following day there appeared alimentary vomiting. A barium meal was eventually followed by vomiting.

The infant was admitted on the third day to the Pediatric Clinic and was found deeply jaundiced, but the physical examination was otherwise negative. There were no hepatosplenomegaly. Dehydration was very moderate.

The direct Coomb's test was found negative.

Hematological findings were as follows:

- RBC 4,400,000 per cu. mm.
- Nucleated B.C. 6,100 per cu. mm.
- Hemoglobin 19 Gm. per cent

**Leucocyte count**

- Polymorph. neutro. 34
- Lymphocytes 45
- Monocytes 10
- Erythroblasts acidoph. 6
- Myelocytes 3
- Plasmocytes 2

Unfortunately, a reticulocyte count was not performed.

In order to prevent dehydration from vomiting, a perfusion was started with 250 ml. of 5 per cent dextrose solution and 50 ml. of normal saline.

The following day, that is four days after birth, serologic studies showed the following:

**Father:** A; B MN ade/ide
Mother: O anti-A anti-B M CDe/cde Her serum is found to contain an "immune" type of anti-A.

Baby: A, M CDe/cde Her serum contains no anti-B and the search for anti-A remains negative.

There was 25 mg. per cent of bilirubin in the infant's serum. Cephalin-cholesterol test (Hanger) was positive after 24 hours. Thymol-Turbidity test: 1 McIaglan unit. There was no detectable hemolysis as indicated by the R.B.C. (4,980,000 cells per cu. mm), and the hemoglobin value (19.5 Gm. per cent).

Clinically there was a certain degree of drowsiness and lack of response to external stimuli. The jaundice was intense as described previously and the vomiting persisted.

An exchange-transfusion using 600 ml. of adult A1 blood was performed through the umbilical vein (still found permeable in its intra-parietal course) and was completed in two hours' time. Heparinization was insured by a single dose of 2,000 units, recalcification by a total dose of 1.9 Gm. of calcium gluconate. Hemostasis of the surgical incision was difficult in spite of local thrombin and general administration of 250 mg. N-methyl-2,3-dihydro-3-hydroxy-5,6-quinone indol-semicarbazone (Adrenoxyl). The umbilical bleeding continued and made necessary a perfusion of 75 ml. whole blood of group A.

On the following morning the baby's condition had considerably improved. Jaundice was still present but vomiting and drowsiness had disappeared.

On the 7th day, that is 72 hrs after the exchange transfusion, the R.B.C. fell from 4,100,000 to 2,655,000 cells per cu. mm, while the anti-globulin test was becoming slightly positive. Transfusion with O whole blood was given in three administrations of 25 ml and brought the R.B.C. back to normal.

At the same time the spleen was felt under the left costal margin and increased in volume from one hour to the other. There was no hematuria or hemoglobinuria.

On the 9th day the R.B.C. had fallen back to 2,600,000 cells per cu.mm. The antiglobulin test was strongly positive, pallor of the skin gradually replaced icterus. The spleen was still increasing. Another transfusion of 25 ml. whole blood was given with a satisfactory, and this time durable, result in the R.B.C.

From that day on the infant recovered gradually and transfusions were given on the 26th, 39th and 40th days to correct the slight anemia.

Serological Findings

(1) Direct anti-globulin tests. As stated above, direct anti-globulin tests were performed and twice found negative before the exchange transfusion. It was positive on the 4th and 7th day and returned to negative on the 14th day. A control using O M CDe-cDE cells, sensi-

<table>
<thead>
<tr>
<th>Serial dilutions of the Coombs serum (Rabbit PR7 K)</th>
<th>5</th>
<th>10</th>
<th>50</th>
<th>100</th>
<th>500</th>
<th>1000</th>
<th>5000</th>
<th>Saline control</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Positive control (0.25 ml O R1, R2 cells + 0.25 anti-D serum)</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
<td>+</td>
<td>±</td>
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<td>-</td>
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<tr>
<td>b) Baby's cells</td>
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<td>(1) day of admission</td>
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<tr>
<td>(2) 4 days after exchange transfusion*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
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<tr>
<td>(3) 7 days after exchange transfusion</td>
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<td>++</td>
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<td>(4) 14 days after exchange transfusion</td>
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* Group A1 blood was advisedly employed in the exchange transfusion.
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TABLE 2.—Mother’s Serum 14 Days Post Partum

<table>
<thead>
<tr>
<th>Tests with</th>
<th>Titers of native serum in saline</th>
<th>Titers after partial neutralization with purified A substance</th>
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<tr>
<td></td>
<td>37 C.</td>
<td>4 C.</td>
</tr>
<tr>
<td>A₁</td>
<td>128</td>
<td>64</td>
</tr>
<tr>
<td>A₂</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>A₁B</td>
<td>256</td>
<td>64</td>
</tr>
</tbody>
</table>

* Titer of the anti-globulin serum with the sensitized cells.

TABLE 3.—Mother’s Serum 24 Days Post Partum

<table>
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* Titer of the anti-globulin serum with the sensitized cells.

tized with a powerful anti-D serum, gave similar reactions thus indicating that the tests performed on the baby’s cells were significant.

(2) The immune properties of anti-A in the mother’s serum. The serum was investigated on the 4th, 14th, and 24th days post partum.

The first sample was small and the presence of immune anti-A was investigated by the method of thermal optimum. The agglutination in saline of the baby’s cells was stronger at body temperature.

On the 14th day a large sample of the mother’s serum was thoroughly studied by three methods:

(1) the thermal optimum

(2) the agglutination in protein media after partial neutralization with A group substances

(3) the indirect anti-human-globulin test after partial neutralization

As test cells, A₁, A₂ and the A₁B cells of the husband were used.

On the 24th day, the same test was performed with A₁ and A₂ cells.

The results are given in tables 1 and 2.

It may be seen that the antibody is undoubtedly immune, but that its specificity was only anti-A₁ in the first sample. In the second sample (24th day), it extended to the agglutinoegen A₂ at any rate with the thermal optimum method and the indirect anti-globulin test.

The agglutination in protein media after partial neutralization remained negative with A₂ cells.

Unfortunately we could not obtain a further sample of serum and our serological investigation came to an end.

DISCUSSION

(A) The Immune anti-A Antibody

The anti-A antibody described in the mother’s serum was undoubtedly immune.

On the 14th day the serum differentiated sharply A₁ and A₂ cells, the latter giving no reaction of the immune type. The A₂ cells were optimally agglutinated...
at 4 C. in saline, did not react in protein media, nor were they sensitized to the anti-globulin reaction after partial neutralization.

This was not true any more with the 24th day serum which at 37 C. agglutinated optimally A2 cells in saline and sensitized them to the anti-globulin serum after partial neutralization. However, the partially neutralized serum gave negative reactions in protein media.

We have previously observed in a case of iso-immunization through a heterospecific transfusion of A1 blood,22 that the change from 4 C. to 37 C. of the thermal optimum occurred quickly for A1 cells and somewhat later for A2 cells. Crawford et al.,8 have demonstrated that immunization against A substance could result in the formation of both anti-A1 (a1) and anti-A (α) immune antibodies. Our case shows that sensitization by an A1 fetus determines also the formation of both antibodies, the speed of appearance thereof being related to the antigenicity of A and A antigens. This fact is in agreement with the von Dungern-Hirszfeld experiment46 which showed in the early days of blood group work, that A1 and A2 cells were quantitatively different, as the former, in suitable amounts, absorbed the anti-A (α) agglutinin of a serum of group B, leaving the anti-A1 (a1) agglutinin as would do a volume 100 times greater of the latter.

Another point of interest is the action of purified blood group substance A, readily inhibiting the anti-A (α) antibody and apparently to a lesser degree anti-A1 (a1). Studies along this line may bring some light on their specificity.

(B) The Negative Direct Coombs Test of the Baby’s Red Cells

Direct antiglobulin test is often negative in infants affected with hemolytic disease due to ABO incompatibility.

This may be ascribed, as stated by Mollison,25 to the “immaturity” of the receptors in the newborn A or B cells and correlated to the very mild hemolytic process.

As shown by Witebsky and Engasser49, 12 about half of the A and B cord cells react in protein media with partially neutralized specific immune sera, in a manner similar to adult A2 cells. We have satisfactorily repeated their observations and extended them to the antiglobulin test. Recently, in a case of iso-sensitization by the antigen B of her fetus, one of our patient's serum, partially neutralized with B blood group substance, sensitized strongly adult B cells to the antiglobulin serum, while it was inactive upon all cord bloods of group B.26

In the present case, the sensitization of the infant’s group A1 cells, which occurred in vivo, could not be demonstrated in vitro, the direct Coombs test remaining negative until A1 adult cells were introduced in the circulation. Exchange transfusion with 600 ml. of adult A1 blood (thus replacing 80 per cent of the blood volume) was followed by in vivo sensitization of these cells (demonstrable in vitro by the antiglobulin test) and subsequent hemolysis.

As the operation had withdrawn 80 per cent, at least, of the antibody from the baby’s circulation, a simple calculation may eventually show that the “resistance” of infant A1 cells to the action of immune anti-A, both in vitro and in vivo, must be great. If it is related to an inverse reciprocal quantity of the agglutinogen at the cell’s surface, it is not understandable why cord cells react as well as adult in ordinary blood grouping with natural anti-A or anti-B, why
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they are just as well capable of absorbing these agglutinins, why, in our case, they reacted strongly at room temperature with natural anti-A\textsubscript{1} sera (group B sera absorbed with A\textsubscript{2} cells).

From these observations we may infer that further investigations are required before we can understand the particular behavior of group A and B cord bloods in the presence of immune anti-A or anti-B antibodies, and in particular the mildness of hemolysis in the disease due to ABO iso-sensitization.

C) The Clinical Picture and the Treatment

There is a great similarity between our case and some recently presented.\textsuperscript{9} The onset of the jaundice was immediate. The bilirubin value was high (25 mg. per cent) and there were slight signs of liver damage. Alimentary vomiting was a very striking symptom which suggested a possibility of pyloric hypertrophy, although this condition is not frequent in girls. We have recently observed the same symptom in a case of hemolytic disease due to anti-B.\textsuperscript{74} There was also a deep and alarming drowsiness.

During the exchange transfusion with A\textsubscript{1} adult blood, there was a progressive improvement of this drowsiness. At the time the operation was completed, the infant had a strong cry and normal movements of the limbs. The vomiting also dramatically disappeared.

The secondary hemolytic anemia appeared 24 hours after the exchange transfusion. It was intense but perfectly controllable with simple transfusions of O blood.

We have developed elsewhere the reason why we choose in exchange transfusion procedures, a blood possessing the specific antigen involved in the iso-immunization process.\textsuperscript{24} It is based upon pathogenic concepts of hemolytic disease ascribing a cytotoxic activity of the hemolytic antibody upon nervous and hepatic cells. It has been shown\textsuperscript{2, 3, 6, 42, 40} that Rh antigens are present in tissue cells and are capable of combination with the anti-Rh antibody. As it is a long known fact that hemolytic sera may equally be cytotoxic,\textsuperscript{4} necrotic lesions of nervous and hepatic cells in hemolytic disease could be reasonably attributed to the anti-Rh antibodies. Soeters\textsuperscript{40} has recently shown that such a mechanism accounts for hepatic lesions (very comparable to that observed in hemolytic disease of the newborn) induced in the guinea-pig by the injection of anti-Forssman sera.

When performing an exchange-transfusion with Rh negative blood, one may theoretically remove all the sensitized Rh positive cells and all the antibody in excess. However such a procedure cannot withdraw the anti-Rh antibody already fixed in the tissues.

By using Rh positive blood we remove indeed the sensitized cells and the excess of antibody and we hope, in surplus, to displace the anti-Rh antibody from its tissular combination by supplying a great excess of fresh antigen. As it is well known that the antigen-antibody complex is reversible in the presence of an excess of antigen, such a hope is not beyond possibility of achievement.

The greatest objection to the use of Rh positive blood for transfusion procedures in hemolytic disease is indeed the poor survival rate of these cells due to their sensitization by the anti-Rh antibody. This objection still holds for simple transfusion.
For three years, we have performed routinely exchange transfusions with Rh positive blood in cases of hemolytic disease due to Rh antagonism. The sensitization of the cells is followed throughout with the direct anti-globulin test. Usually the test is negative and remains negative after the procedure. Although sensitization cannot be demonstrated anymore in vitro by the Coombs test, the survival rate of the transfused cells may be shortened and a progressive anemia may be seen in the first weeks. This is always perfectly controllable by simple transfusions with Rh negative blood.

Since our results with this method were good in cases of Rh sensitization, we were induced, in the present case, to use A1 blood for exchange transfusion. Aside from a temporary hemolysis easily controlled by simple transfusion therapy, the recovery was uneventful, and alarming symptoms were relieved. Thus the use of A1 blood “incompatible” with the passively transferred antibody did not have any serious consequences. It gave us the possibility to observe a very important fact: the antiglobulin test was negative before treatment and became strongly positive when adult A1 cells were introduced in the infant’s circulation.

**SUMMARY**

1. The authors report clinical, hematological and serological data in a case of A1 iso-sensitization of pregnancy.
2. The mother’s serum displayed immune characters specific to A1 cells immediately after delivery. On the 24th day post partum the specificity extended to A2 cells.
3. The disease exhibited by the infant was very mildly hemolytic. It was marked by a deep jaundice, repeated alimentary vomiting and a progressive state of drowsiness. There was no anemia. The direct anti-globulin test was negative.
4. It is shown that the mildness of the hemolytic process in cases of placental transfer of immune anti-A or anti-B into the incompatible A or B fetus is probably dependent upon a peculiar “resistance” of the fetal cells. This may be demonstrated in vivo and in vitro. In the present case replacement transfusion with A1 adult blood resulted in its in vivo sensitization, detectable by the antiglobulin test and eventually leading to hemolytic anemia.

**SUMMARIO IN INTERLINGUA**

1. Le autores reporta datos clinic, hematologic, e serologic in un caso de pregnantia con iso-sensibilisation a A1.
2. Immediatemente post parto le sero del matre exhibiva characteristicas immun specific a cellulas A1. Le 24e die post parto le specificitate se habeva extendite a cellulas A2.
3. Le morbo exhibite per le infante esseva levemente hemolytic. Illo esseva distinguite per forte ictero, repetite vomiturition alimentari, e un stato progressive de somnolentia. Il non habeva ulle anemia. Le reaction anti-globulinic directe esseva negative.
4. Le autores demonstra que le leve grado del processo hemolytic in casos de transferentia placental de anti-A o anti-B immun a in le incompatibile feto A o B depende probablemente de un characteristic “resistentia” del cellulas fetal. Isto pote esser demonstrate “in vivo” e “in vitro.” In le presente caso trans-
fusiones de reemplaciamiento con sanguine adulto A₁ resultava in su sensibilisation in vivo lo que esseva detegibile per medio del reaction antiglobulinic. In le curso del tempore illo resultava in anemia hemolytica.

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