Unexpected Antibody Found in Pregnant Women, and Haemolytic Diseases in Newborn Trial of Blood Transfusion into Rh(E)-Negative Women

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Abstract

Since January, 1979, we examined all pregnant women visiting our hospital on anti-erythrocyte unexpected antibodies in order to detect haemolytic disorder in the newborn (HDN) as early as possible. Among the 4,748 pregnant women examined, antibodies could be found in 147 cases (3.1%). Anti-Le antibodies were found to be the most frequent, and anti-E formed the majority of anti-Rh antibodies detected. Blood transfusion seemed to play a great role in the production of anti-E in our examined women. Among the seven Rh(E)-incompatible pregnant cases at our hospital, two were so severe as to need exchange transfusion therapy. Therefore, trials were made ; i. e. Rh(E)-negative red blood cell preparations were transfused into the same-blood-type women under forty years of age requiring blood transfusion. We had to prepare 6.9 units of Rh(E)-negative red blood cell preparations per day. The amount was within the capacity of our transfusion unit. Therefore, Rh(E)-negative red blood cell preparations should be used for blood transfusion into Rh(E)-negative women under forty years of age who may experience future pregnancy. Moreover, not only anti-Rh(D) immunoglobulin preparations, but anti-Rh(E) preparations should be developed, particularly in Japan.

Introduction

In pregnant women, anti-erythrocyte unexpected antibodies have been detected more frequently than in ordinary donors^{1) 2) 3) 4)}. These antibodies, especially of the IgG class, can cause haemolytic diseases in the newborn (HDN) due to incompatibility of blood types

between mother and fetus by a transfer of the IgG antibodies from the mother to the fetus via the placenta, resulting in a shortening of the infant's red blood cell life-span. HDN due to Rh-incompatibility are clinically most important because of its high incidence and its severity, and, among HDN due to Rh-incompatibilities, those due to anti-C, -E, -c, or anti-e have been less frequently observed than those due to anti-D⁵⁾.

However, HDN caused by anti-D is less frequent in Japan as only about 0.5% of the Japanese population are Rh(D) negative, in contrast to the 15% found among the white races^{5) 6)}. Moreover, anti-Rh(D) immunoglobulin products have been recently introduced into clinics to lower further the frequency of HDN caused by anti-D⁷⁾. Consequently, anti-E is now playing the most critical role in the cause of HDN in Japan^{3) 4)}.

In order to detect HDN as early as possible, we have since January 1979, examined all pregnant women visiting our hospital on the anti-erythrocyte unexpected antibodies. We have also made attempts to use Rh(E)-negative red blood cell preparations for transfusion into Rh(E)-negative women under forty years of age in order to prevent the incidence of anti-E, in the event of future pregnancy.

The results are as follows :

Materials and Methods

Figure 1 shows our system used since January 1979 for detecting HDN due to blood type incompatibility between mother and fetus or infant. All of the pregnant women consulting our hospital were examined on their blood type and anti-erythrocyte antibodies twice during pregnancy, i.e. in the 2nd and 8th months. The pregnant women, who showed antibodies, received more precise examinations for antibody identification and were followed up unto the

Figure 1 Flow chart of examination system in our hospital for detection of blood type incompatibility between mother and fetus or newborn baby

delivery. As to the Rh(D)-negative pregnant women, the examinations were repeated periodically to detect the appearance of the antibody as early as possible. At the delivery, those who had been Rh(D)-negative or had been found to have some antibodies were examined by several tests shown in **Table 1** IV to detect blood type incompatibility between mother and infant. And, infants transported to our hospital from other hospitals due to severe hyperbilirubinemia were also similarly examined.

Table 1 Anti-erythrocyte antibodies detected in pregnant women
(Jan. 1979 - Apr. 1984)

Total number examined : 4748 cases

Number of cases having antibody : 147 cases (3.1%)

pregnancy 2nd month	(-) (+)	(-) (+) (-) (+)	/ /
8th month	/ /	(-) (+) (+) (-)	(-) (+)
no. of cases (%)	1714 67 (96.2) (3.8)	2546 45 29* 2 (97.1) (1.7) (1.1) (0.1)	341 4 (98.8) (1.2)
total	1781	2622	345

(* anti-Le^a 9, -P, 5, -Le^b 2, -E 2, -Bg^a 1, -JK^b 1, -E+c 1, undetermined 8)

The examinations on the blood type included, among others, ABO and Rh-Hr type. Screening for anti-erythrocyte antibodies was done by means of the saline, albumin Coombs, and bromelin methods⁸⁾ using panel cells and Surgiscreen (Ortho Diagnostic Systems Inc.). In order to identify the detected antibodies, Resolve A,B (Ortho Diagnostic Systems Inc.) were used, and to estimate the titers of the antibodies, sera diluted with saline were examined using the four methods mentioned above for antibody identification. The direct Coomb's test was also carried out in the incompatible infants using polyspecific antihuman serum, monospecific antihuman IgG, and C₃ (Ortho Diagnostic Systems Inc.). The eluates from red blood cells of the infants were obtained by means of the heat technique⁹⁾ in the ABO incompatible cases, and by the chloroform-trichloroethylene method¹⁰⁾ in the Rh incompatible ones.

Results

1. Anti-erythrocyte antibodies found in pregnant women

Table 1 shows the results obtained from 4,748 pregnant women examined during the past five years (Jan. 1979 - Apr. 1984). Among these women, some of the antibodies could be found in 3.1% (147/4,748) while they could only be found in 2.1% (686/32,497) among all individuals examined (ordinary patients, donors, and pregnant women) at our hospital during nearly the same period. Among the 2,622 cases who could be re-examined in the

2nd and the 8th months during pregnancy, the findings were negative in 2,546 (97.1%) and positive for some antibodies in 76 cases (2.9%). Conversion from negative to positive was seen in 29 cases (1.1%); i.e. anti-Le^a 9, -P₁ 5, -Le^b 2, -E 2, -Bg^a 1, -JK^b 1, -E+c 1, undetermined 8 cases, at the 8th month's examination, while a seroconversion to negative could only be found two anti-Le^a cases.

Figure 2 shows the respective percentages of anti-erythrocyte antibodies derived from 147 pregnant women. Anti-Le antibody was found in 68 cases, covering about half (46.3%) of all positive cases. As to anti-Rh, anti-E (4.8% of all) constituted the majority (7/8 cases). Besides, anti-P₁, -M, -JK^b, and anti-Bg^a could be detected, but the antibodies supposed able to induce HDN; i.e. anti-Rh (8 cases), IgG anti-M (3 cases), and anti-JK^b or anti-Bg^a (3 cases), occupied only 9.5% of all the positive cases.

As to the causes supposed to have induced the anti-E in the 21 women found at our blood transfusion unit from Jan. 1979 to Apr. 1984, the pregnancy seemed to be the cause in 11 cases (52.4%), and blood transfusion in 6 cases (28.6%), but no cause could be inferred in the remaining 4 cases. As the opportunity of receiving blood transfusion is much less than that of receiving pregnancy, blood transfusion may play the most sinister role in the production of anti-E antibody.

Figure 2 Classification of anti-erythrocyte unexpected antibodies derived from 147 pregnant women

2. Anti-erythrocyte antibodies found in infants born from blood type incompatible mothers and in those having received exchange transfusion (Figure 3)

Among 298 infants born from blood type incompatible mothers, antibody was detected in 82 cases (27.5%), 9 of whom (11%) needed exchange transfusion therapy. Fifty-nine (72 %) of these 82 positive cases were ABO incompatible, among whom exchange transfusion was carried out in 2 cases (2.4%) with IgG anti-A, and in 4 cases (4.9%) with IgG anti-B. On the other hand, 9 infants (11%), all born from Rh(D)-positive mothers, showed Rh incompatibility. Among them, each one having anti-E, anti-E+c, or anti-C+e, all being other than anti-D, needed exchange transfusion therapy.

Figure 3 Classification of antibodies found in infants born from blood type incompatible mothers and in those having received exchange transfusion therapy

3. Rh(E)-incompatible cases

Figure 4 shows seven Rh(E)-incompatible pregnant cases which we had experienced at our hospital during the past five years, including some infants transported to us from other

case	titer of antibody found in mother at delivery	neonatal			therapy
		direct Coombs	antibody	bilirubin	
B. T.	anti-E	4+	E	mg/dl 37.6	exchange transfusion
B. Y.	anti-c anti-E	4+	E+c	18.2	"
M. M.	anti-E	2+	E	11.1	phototherapy
S. Y.	anti-c anti-E	2+	E	12.5	"
K. S.	anti-E	1+	E	/	(-)
K. T.	anti-c anti-E	4+	E	16.3	phototherapy
B. T.	anti-c anti-E	3+	E+c	15.8	"

1:1 1:2 1:4 1:8 1:16 1:32 1:64 1:128 1:256 1:512 1:1024

Figure 4 List of Rh(E)-incompatible cases (1979~1984)

hospitals due to severe hyperbilirubinemia. The antibodies detected from mothers at delivery were anti-E alone in 3 cases and anti-E+c in 4 cases. Among these 7 cases, 2 cases (Cases 1, 2) were so severe as to require exchange transfusion therapy, 4 cases (Cases 3, 4, 6, 7) could be treated with phototherapy alone, and the remaining one (Case 5) required no therapy. The antibody titers derived from the maternal serum did not always correlate with the severity of the haemolytic diseases of their infants; e.g., the antibody titer of the mother was very high (1:1024) in case 6, while her infant's haemolytic disease could be successfully treated with phototherapy alone without resort to exchange transfusion. In all of these 7 cases, the ABO blood type were compatible between mother and infant.

4. Trial of blood transfusion of Rh(E)-negative red blood cell preparations into Rh(E)-negative women

The daily amount of Rh(E)-negative red blood cell preparations necessary for blood transfusion in our hospital as calculated from the data of the past two years was; mean daily number of Rh(E)-negative women under 40 years of age needing red blood cell transfusion in our hospital=mean daily number of all patients needing red blood cell transfusion in our hospital $\times$ frequency of women under 40 years of age among these patients (0.247) $\times$ frequency of Rh(E)-negatives in Japanese (0.512)=1.3 patients per day, mean daily required units of Rh(E)-negative red blood cell preparations in our hospital=mean daily number of Rh(E)-negative women under 40 years of age needing red blood cell transfusion in our hospital (1.3) $\times$ mean requested units per patient in our hospital (5.3)=6.9 units. Thus,

we had to prepare 6.9 units of Rh(E)-negative red blood cell preparations per day. The amount was within the capacity of our transfusion unit. Therefore, trials were made to transfuse the same blood type red blood cell preparations toward Rh(E)-negative women under forty years of age requiring blood transfusion during the two month period June through July, 1982. During this period, we had to prepare 5,040 units for 803 patients in total. Among these units, 385 units (7.6%) were given to 99 female patients under forty years of age, and, among them, 172 units (3.4%) were for 40 Rh(E)-negative patients. This time, preparations such as platelet were excluded from the trial due to the difficulty in making such Rh(E)-negative preparations. If these were also to be included in this trial, 734 units (14.6%) would be required for female patients under forty years of age out of 5,040 units in total, and 328 (6.5%) of them were to be transfused toward the Rh(E)-negatives. As it was, in order to prepare the 172 units of Rh(E)-negative red blood cell preparations actually used, 437 units had to be checked for Rh(E) factor, i.e. 1:2.5.

Discussion

In the literature, the unexpected anti-erythrocyte antibodies are reported to be found in percentage five to ten times higher in pregnant women than in ordinary donors. As mentioned above, our blood transfusion unit could detect such antibodies in 3.1% of our pregnant women. The frequencies reported from other institutions in Japan range from 1.40 to 1.62 % in pregnancy^{1) 2) 4)}, and 1.86% at delivery³⁾. As to the kinds of the antibodies derived from our pregnant women, IgG antibodies which might become responsible for HDN occupied only 9.5% of all these antibodies. Among these IgG antibodies, the anti-Rh were the most common (5.4% of all), anti-E (4.8% of all) constituting the majority of these anti-Rhs. One report revealed the same trend that anti-Rhs were the most common antibodies (37.8% of all) found in Japanese pregnant women, and, among these, anti-E were in majority (18.3% of all) followed by anti-D (11.9% of all)⁴⁾. Another report expressed the similar findings that, among the IgG antibodies covering 26.5% of all antibodies in Japanese pregnant women, anti-E was the most common³⁾.

On the other hand, in the white races, such anti-erythrocyte antibodies are reported to be detectable in 2.3~5.7% of pregnant women, anti-D being the most frequent among these (94.7%)⁵⁾. This difference may depend upon the very low frequency (0.5%) of Rh(D)-negatives seen in the Japanese, contrasting with the 15% seen in the white races⁵⁾⁶⁾. Although, anti-D accounted for 93% of the antibodies causing haemolytic disease in white infants up to 1970⁵⁾, this percentage has been reduced to 57% (1976~1980) in concomitance with the clinical introduction of the anti-Rh immunoglobulin⁷⁾. In Japan, too,

HDN due to anti-D has been reduced, but on the other hand the importance of anti-E as a cause of HDN is increasing. Although the HDN caused by anti-D has been reported to be more severe than those due to other anti-Rh antibodies¹¹⁾, our four cases of the HDN caused by anti-c found concomitantly with anti-E also tended to become severe. Although it has been reported that the HDN due to anti-E does not generally show severe clinical symptom, it should be worth mentioning that two of our seven anti-E incompatible cases necessitated exchange transfusion. This finding may be partly attributed to the fact that severe HDN patients are often transported to our NICU from surrounding hospitals, but the same trend was also recognized in our own ABO incompatible HDN cases. Although most authors report that HDN due to IgG anti-A or -B require exchange transfusion very rarely (0.33%)¹²⁾, 7.3% of our ABO incompatible HDN cases did necessitate this treatment. On the other hand, one of our Rh(E)-incompatible HDN could be cured simply by means of the phototherapy in spite of the very high anti-E titer in the maternal serum. Concerning the correlation between serum antibody titer and severity of HDN, it has been reported that, in cases with anti-D of IgG₁ subtype, the severity of the infant's HDN paralleled the antibody titer in the maternal serum, while, in cases caused mainly by IgG₃ anti-D, such a correlation was obscure¹³⁾. Therefore, we should hereafter examine the IgG subtype of the anti-E antibody in each HDN case due to Rh(E)-incompatibility. Moreover, anti-Rh antibodies can be produced in ABO-compatible mothers at a frequency eight times as high as that in ABO-incompatible ones¹⁴⁾. In fact, all of our seven Rh(E)-incompatible cases had compatible ABO blood types. As to the causes of anti-E antibody production, blood transfusion seemed to be quite as important to Rh(E)-incompatible pregnancy. Therefore, Rh(E)-negative red blood cell preparations should be used for transfusion toward Rh(E)-negative women under forty years of age who may become pregnant. On the other hand, the development of anti-Rh(E) immunoglobulin preparations should be urged, more particularly in Japan, as the less urgent anti-Rh(D) preparations are already available.

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