THE AGGLUTININ ANTI-M ASSOCIATED WITH PREGNANCY

REPORT ON TWO CASES

BY

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In the following two cases of pregnancy the agglutinin anti-M was detected.

Case 1

Mrs. A, aged 27, was expecting delivery of her first child on December 22, 1946. The serological findings are shown in the Table.

May 20.—Mrs. A : Group A Rh-negative (cde/cde). W.R. negative. No abnormal antibodies active at 37° C.

August 1.—Mr. A : Group O Rh-positive. His cells were not agglutinated at 37° C. by his wife's serum (saved from May 20 and stored at -20° C.).

December 3.—Mrs. A : Serum contains an antibody active at 37° C. agglutinating 13 different red cell suspensions (two Rh-negative and 11 Rh-positive), each known to contain the antigen M, but did not agglutinate seven different red cell suspensions all lacking in the antigen M.

December 10.—Mrs. A : Red cells—Group A Rh-negative N,P. Serum—action of antibody identical with that of a known anti-M when tested against 100 unknown cells. Titre of antibody 128 at 37° C., 20° C., and 4° C., with a weak reaction at 37° C. and a visual reaction at 4° C.

December 16.—Mrs. A was delivered of an apparently healthy child showing no clinical signs of haemolytic disease of the newborn. The placenta was normal in appearance and weight. Baby A (cord blood) : Apparently Group A Rh-negative ; M,N,P. The direct Coombs test was negative. The child's cells were strongly agglutinated at 37° C. by its mother's serum. Haemoglobin at 24 hours was 162% Haldane. Mrs. A : Findings as before. The titre against the same M-positive cell 64 at 37° C. (weak reaction), 64 at 20° C., and 128 at 4° C. (visual reaction). Mr. A : Group O Rh-positive (cDe/cde) ; M,N. His cells were strongly agglutinated at 37° C. by his wife's serum.

December 23.—Mother and child were discharged from hospital. The child was fully breast-fed and clinically normal.

Table Showing the Serological Findings in Family A

Date	Mrs. A	Mr. A	Baby A
20/5/46	Group A Rh-negative (cde/cde). No abnormal antibodies at 37° C.	—	—
1/8/46	—	Group O Rh-positive. Cells not agglutinated by wife's serum	—
3/12/46	Antibody present active at 37° C.	—	—
10/12/46	Group A Rh-negative ; N,P. Antibody identical with anti-M against 100 unknown cells. Titre: 1: 128 at 37° C. (weak reaction); 1: 128 at 20° C.; 1: 128 at 4° C. (visual reaction)	—	—
16/12/46	Titre: 1: 64 at 37° C. (weak reaction); 1: 64 at 20° C.; 1: 128 at 4° C. (visual reaction)	Group O Rh-positive (cDe/cde); M,N. Cells agglutinated at 37° C. by his wife's serum	Group A Rh-negative ; M,N,P. Coombs's test negative. Cells agglutinated at 37° C. by maternal serum
5/4/47	Titre: 1: 8 at 37° C.	—	Group A Rh-negative ; M,N,P

April 5, 1947.—Baby A : Aged 3½ months. Examined by paediatrician and judged to be normal in all respects. Blood typed as Group A Rh-negative ; M,N,P. Mrs. A : Anti-M still active at 37° C. with a titre of 8.

Case 2

Mrs. B, a 3-gravida, was admitted to the puerperal sepsis ward on March 13, 1947, three days after delivery, requiring transfusion. Blood was sent for Rhesus typing and cross-matching.

March 14.—Group O Rh-negative (cde/cde) ; N. No abnormal antibodies were demonstrable at 37° C., but at 20° C. and 4° C. an antibody was present, behaving as an anti-M with a titre of 2. Cross-matching was carried out at 37° C. and 4° C., and Group O Rh-negative N blood was transfused satisfactorily.

March 26.—Infant B : Aged 15 days. Clinically normal, having shown no signs of haemolytic disease of the newborn since birth. Blood Group O Rh-positive (CDe/cde) ; M,N. The direct Coombs test was negative. The child's cells were strongly agglutinated by its mother's serum at 20° C. but not at 37° C. Mr. B : Group A Rh-positive (CDe/cde) ; M. His cells were strongly agglutinated by his wife's serum at 20° C. but not at 37° C.

Discussion

The findings of an abnormal antibody in maternal sera during pregnancy may generally be taken as indicating iso-immunization, with the future possibility of haemolytic disease of the newborn. It is our contention that the detection of anti-M in maternal serum during the course of pregnancy or the puerperium should not be considered as having the same significance as the detection of such abnormal antibodies as the Rhesus iso-agglutinins. In fact, anti-M, like anti-P (Dunsford, 1949), is to be considered as a naturally occurring antibody, comparable with anti-A or anti-B, but of rarer occurrence.

The literature contains few references to the iso-agglutinin anti-M. Wiener and Forer (1941) and Wiener (1942) have reported what appears to be immune anti-M in two cases following incompatible transfusion, but such an occurrence is so rare and anti-M so weak an antigen in man that M and N factors are ignored in normal transfusion work. Anti-M in association with pregnancy has been reported in only two cases (Paterson *et al.*, 1942 ; McFarlane, 1945), while we have knowledge of three more recently discovered cases. De Kromme and Van Der Spek (1948) have reported a case in which it was suggested that anti-N was associated with haemolytic disease of the newborn, but it was not investigated fully nor was the association proved.

In the cases of Paterson *et al.* and McFarlane and in our Case 2 anti-M was present as a typical cold agglutinin active at 20° C. and 4° C. In no case was there evidence of haemolytic disease of the newborn. Following these three cases, that of our Case 1 is of additional value in that the antibody was strongly active at body temperature with a titre of 128, but again there was no evidence of haemolytic disease of the newborn.

It is believed that Rh is a stronger antigen in man than M (McFarlane, 1945), and if anti-M and anti-Rh are produced by a similar process—namely, iso-immunization—it would be reasonable to suppose that Group N, Rh-negative multigravidae who have borne M or MN Rh-positive children, and have produced anti-M, should also have produced anti-Rh. McFarlane's case and our Case 2 both fall into this category, but in neither case was anti-Rh detected. This suggests that the anti-M present was a natural and not an immune antibody. It will be appreciated that a case reported by Wiener and Forer (1941) in

which both anti-M and anti-Rh were produced by an incompatible transfusion, and another discovered by Stratton (personal communication), with both antibodies present in pregnancy, do not negative this hypothesis.

Secondly, clinical and serological manifestations of haemolytic disease of the newborn were entirely absent in the case of Mrs. A, in whom the antibody was known to have increased in titre during the pregnancy and to have become active against the cells of the foetus and its father at body temperature. Similar findings with an immune iso-agglutinin are almost invariably followed by affection of the newborn.

Finally, a technique of differentiating immune and naturally occurring antibodies was applied to these sera. This technique, described by Boorman and Dodd (1947), is the comparison of the titre of the agglutinin in saline with the titre of the agglutinin when human serum, lacking the antigen to the antibody under examination, is used as diluent in place of saline. Boorman and Dodd found enhancement of titre in serum in which immune antibodies were present, whereas naturally occurring antibodies showed no enhancement. In the cases of Mrs. A and Mrs. B there was no evidence of titre enhancement, further confirming the theory that these anti-M agglutinins were of natural occurrence.

Reason must be given why in the case of Mrs. A the titre of the antibody rose during the intrauterine life of the foetus if the antibody was of natural occurrence. This phenomenon has been reported by Jonsson (1936) and others in the case of naturally occurring anti-A and anti-B, and not infrequently observed by one of us (I.D.). Similar observations have been made in Rh-negative women who, having produced Rh antibodies in previous pregnancies, reproduce these antibodies in increasing titre in a subsequent pregnancy, even though carrying a normal Rh-negative foetus. This can be explained by the supposition that there has been a non-specific stimulation of already existing antibodies during the course of pregnancy.

In the case of Mrs. A an explanation of the alteration of the antibody activity so that agglutination of the infant's and of the father's cells occurred at 37° C. is found in the work of Kettel (1930), who proved that the thermal amplitude of a cold agglutinin—e.g., anti-M—is directly proportional to its titre. The titre of 1:128 of Mrs. A's anti-M is the first reported to have reached sufficient strength to react at body temperature.

Tovey (1944) has suggested that anti-M or anti-N agglutinins will appear only rarely in the serum of a mother who is carrying a baby of dissimilar M or N type. This he suggests is due to the pronounced non-specific absorptive capacity of human tissues for these iso-agglutinins. This may explain why Mrs. A's anti-M, though active at 37° C., did not cause haemolytic disease of the newborn.

Summary

Two cases in which the agglutinin anti-M was associated with pregnancy are reported, and the literature on the subject is reviewed.

Reasons are suggested for considering these anti-M agglutinins to be naturally occurring antibodies.

The relation of anti-M to the aetiology of haemolytic disease of the newborn is discussed.

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Group Reference Laboratory, was kind enough to confirm the findings of Mrs. A's serum.

Note.—An anti-M with the same thermal amplitude as Mrs. A's has been reported to us by Dr. Tovey, of the Regional Blood Transfusion Laboratory, Bristol, and one of us (I.D.) has recently found another anti-M associated with pregnancy, in which case the child, though M-positive, was clinically normal.

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THE RHESUS FACTOR D^u

A NOTE ON ITS PRACTICAL IMPORTANCE

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Rh testing is being undertaken in many laboratories either as a test in antenatal cases or for selecting Rh-negative blood donors. Fisher has suggested that the reactions of the various Rh types should be explained in terms of three pairs of allelic genes, C-c, D-d, and E-e, controlling the presence of the corresponding antigens.

Additional alleles, C^w and D^u, have been detected at the C-c and D-d loci, and it is with D^u at the D-d locus that this paper deals. This theory is fully explained by Mollison, Mourant, and Race (1948).

The D antigen is the most important of the Rh antigens, and a person is now referred to as being "D-positive" or "D-negative" according to whether his erythrocytes do or do not possess the D antigen. In the Manchester area there are about 18% of D-negative and about 82% of D-positive persons, so that, if the rarer subgroups are excluded from our consideration for the moment, it will be seen that this classification roughly corresponds to "Rh-positive" and "Rh-negative."

The D^u Group

When blood cells were being typed for Rh it was noticed that some anti-Rh (anti-D) testing sera reacted with certain blood samples positively, whereas others, apparently equally good testing sera, failed to react with these cell samples. Further examination of these cells revealed that they reacted with up to 45% of anti-D sera only; these cell samples may of course also react positively, or negatively, with anti-C and anti-E sera in the usual way. This special type of D antigen was tentatively called D^u (Stratton, 1946); subsequently cells of types CD^ue/cde, cD^ue/cde, and cD^uE/cde were discovered and recognized (Race, Sanger, and Lawler, 1948a, 1949b; Stratton and Renton, 1948).

It was later found that some types of D^u cell were so weak in their reactions that they would give saline agglutination with no anti-D typing sera, or in some cases with only a very few. Nevertheless, it was found that these cells would