Irregular Isoagglutinin Reactions Encountered in a Tropical Area[1]

By MERCEDES V. TORREGROSA and EDUARDO MONTILLA

From the Blood Bank of Civilian Defense, School of Tropical Medicine, San Juan, Puerto Rico

BLOOD BANKS are playing a role of ever increasing importance in present-day medicine, the prevention of transfusion reactions being one of their main functions. Cross-matching of the bloods of donor and recipient, previous to transfusion, constitutes a safeguard to the life of the patient as such a test reveals not only those major incompatibilities due to a difference in group but also those caused by the presence of atypical isoagglutinins.

In a series of 1,016 cross-matching tests (652 for whole bloods and 364 for red blood cell suspensions) performed in the Blood Bank at the School of Tropical Medicine, there were given 553 O, 361 A, 87 B, and 15 AB bloods. Four of these bloods contained unusual isoagglutinins, and it is the purpose of this paper to discuss them in detail.

ANTI–RH AGGLUTININS

In their study on the agglutinable factors of the human blood, Landsteiner and Wiener[2] demonstrated the presence of an unknown agglutinogen in the red blood corpuscles of 85 percent of the population. They called this agglutinogen the Rh factor, because it was first discovered when trying out sera prepared by immunizing rabbits with the cells of Rhesus monkeys. Those individuals whose blood did not contain this agglutinogen were designated as Rh negative. It was furthermore shown[3] that Rh negative persons, receiving transfusions from Rh positive individuals, and Rh negative pregnant women, bearing an Rh positive foetus, may become isoimmunized and develop agglutinins against the Rh factor. Such isoagglutinins are capable of producing a hemolytic transfusion reaction if Rh positive blood is transfused into the person possessing them.

---

1. Received for publication June 23, 1943.

2. K. Landsteiner and A. S. Wiener, "An Agglutinable Factor in Human Blood Recognized by Immune Sera for Rhesus Blood," *Proc.Soc.Exp.Biol.& Med.*, 43:223, 1940.

3. K. Landsteiner and A. S. Wiener, "Studies on an Agglutinogen (Rh) in Human Blood Acting with Anti-Rhesus Sera and with Human Iso-Antibodies," *J.Exp.Med.*, 74:309, 1941.

A. S. Wiener, "Hemolytic Reactions Following Transfusion of Blood of the Homologous Group. Further Observations on the Role of the Rh Property Particularly in 3 Cases without Demonstrable Antibodies," *Arch.Path.*, 32:227, 1941.

In the case of an Rh negative pregnant woman, bearing an Rh positive foetus, the anti-Rh agglutinins passing through the placenta may bring about a hemolytic process in the foetal blood resulting in different manifestations of erythroblastosis foetalis.[4] Ample statisical and experimental data support this theory. However, Levine *et al*[5] have wisely pointed out that isoimmunization to the Rh factor is not the only cause of erythroblastosis foetalis. Wiener and Peters[6] are of the opinion that preformed anti-Rh antibodies must be present in order for the mother to become isoimmunized; this explains why some Rh negative women may deliver normal Rh positive infants.

Dr. M. Fernández Fuster, of the Fajardo District Hospital, supplied us with blood from a woman who had delivered a still-born erythroblastic foetus two months previously. Her blood was found to belong to group O and to be Rh negative; her husband's blood was of group O and Rh positive. Atypical isoagglutinins were demonstrated in her serum active against the husband's blood as well as against several other group O, Rh positive bloods. No agglutination occurred when the serum was cross-matched with O, Rh negative cells. The tests were carried out in accordance with the modified Levine[7] test tube technique, which is more sensitive in detecting weak agglutinin reactions like those produced by the Rh factor. Compatibility tests were performed at room temperature, 37° C and 5° C, as suggested by Wiener and Peters,[8] since anti-Rh agglutinins vary in their optimal temperatures.

In the case under consideration the blood of the infant was not available. The father proved to be Rh positive and, since this trait is inherited as a Mendelian dominant,[9] it seems highly probable that the foetus was also Rh positive. Isoimmunization of the mother during pregnancy would have explained the phenomenon of erythroblastosis foetalis as well as the presence of the irregular isoagglutinins. She should be transfused only with group O, Rh negative blood while the immune isoagglutinins persist in her serum.

4. P. Levine, L. Burnham, E. M. Katzin, and P. Vogel, "The Role of Iso-immunization in the Pathogenesis of Erythroblastosis Foetalis," *Am.J.Obst.& Gyn.*, 42:925, 1941.
5. P. Levine, E. M. Katzin, and L. Burnham, "Iso-immunization in Pregnancy. Its Possible Bearing on the Etiology of Erythroblastosis Foetalis," *J.A.M.A.*, 116:825, 1941.
6. A. S. Wiener and R. Peters, "Hemolytic Reactions Following Transfusion of Blood of the Homologous Group with 3 Cases in Which the Same Agglutinogen Was Responsible," *Ann. Int.Med.*, 13:2306, 1940.
7. P. Levine, "Role of Iso-immunization in Transfusion Accidents in Pregnancy and in Erythroblastosis Foetalis," *Am.J.Obst.& Gyn.*, 42:163, 1941.
8. A. S. Wiener and R. Peters, *op. cit.*
9. K. Landsteiner and A. S. Wiener, *op. cit.* (3)

Immune anti-Rh agglutinins were demonstrated in the serum of a woman two years after delivery of an erythroblastic infant,[10] yet statistical data show that these isoagglutinins usually disappear much faster.

ANTI–A AGGLUTININS

Groups A and AB have been divided into subgroups A_1, A_2, A_3, and A_4 and A_1B, A_2B, A_3B, and A_4B.[11] There exists a divergence of opinion as to whether these subgroups represent qualitative or quantitative differences; however, isoimmunization of one subgroup against another seems to point towards a qualitative antigenic difference.[12] A small percentage of individuals belonging to groups A and AB possesses atypical isoagglutinins in the serum which react against the blood of another subgroup.[13] In cases where blood of one subgroup is transfused into persons belonging to another, as an A_1 blood being given to an A_2 individual, no reaction occurs. In certain rare cases, presumably in those persons possessing preformed irregular agglutinins anti-A_1, a mild reaction may occur[14] with a corresponding rise in the titer of anti-A_1 agglutinins. If another transfusion is given with an A_1 blood, a serious reaction may then be produced due to the presence of the strong anti-A_1 agglutinins. The same holds true of an A_1 individual receiving an A_2 blood, the recipient in this case developing anti-A_2 agglutinins. These isoagglutinins have also been called anti-O because they are even more active against O bloods.

We had the opportunity of studying the presence of an anti-A_2 agglutinin in A_1 blood, where an isoagglutinin capable of clumping A_2 cells and also active against the cells of several O bloods was present. Two A_1 bloods proved compatible. The patient was successfully transfused with A_1 blood but there was doubt as to whether a transfusion with an A_2 or an O blood might not have proved harmful because of the presence of the strong irregular isoagglutinin described. It was impossible to determine whether this was a natural

10. R. Fisk and A. Foord, "Observations on the Rh Agglutinogen of Human Blood," *Am.J. Clin.Path.*, 12:545, 1942.
11. F. Shiff and W. C. Boyd, "*Blood Grouping Technique*" (New York: Interscience Publications, 1942).
12. A. S. Wiener, "Subdivisions of Groups A and AB. II. Immunization of A_2 Individuals against A_1 Blood, with Special Reference to the Role of Subgroups in Transfusion Reactions," *J.Immunol.*, 41:181, 1941.
13. K. Landsteiner and P. Levine, "Isoagglutinin Reactions of Human Blood Other than those Defining the Blood Groups," *J.Immunol.*, 17:1, 1929.
14. A. S. Wiener, *op. cit.* (12).

or an immune isoagglutinin, the patient having never received a blood transfusion as far as was known. She had recently delivered a full-term child. The subgroup of the foetus was not determined yet it may have been the source of isoimmunization.

COLD AGGLUTININS ACTIVE AT ROOM TEMPERATURE

Certain sera contain nonspecific agglutinins which, when mixed at low temperatures (0° to 5° C) with human red blood cells of the different blood groups, produce an agglutination of the blood cells; agglutination disappears upon heating. This phenomenon has been called cold agglutination. Since sera containing such agglutinins may agglutinate (at ice-box temperature) the cells of the same individual from whom they originate, these agglutinins have also been called autohaemagglutinins.[15] The presence of these nonspecific autoagglutinins is a normal physiological phenomenon; however, in certain diseases such as paroxysmal hemoglobinuria, Reynaud's disease, trypanosomiasis, severe anemia, sepsis, hemolytic icterus, and other conditions, autoagglutinins are present active in high dilutions and are sometimes demonstrable at room temperature.[16] More statistical data would be very valuable on this subject to determine whether there is any specific cause for enhancing this particular serological mechanism.

A blood with autoagglutinins, obtained from a case of puerperal sepsis with severe anemia, showed the agglutinin reactions outlined in Table 1. The patient belonged to group AB, as determined by the use of immune rabbit anti-A and anti-B sera; the subgroup, though, was not known at the time as there was no absorbed B serum available. The serum of an AB blood would not agglutinate the blood cells of other groups, but the agglutinin reactions of this patient's serum with AB, A, B, and O cells, as well as with the patient's cells, revealed the presence of an irregular isoagglutinin. This agglutinin was very active at 5° C, producing complete clumping of the cells of all blood groups and of the patient's as well. A weak agglutination of the red blood cell suspensions was still evident at room temperature, but at 37° C the different mixtures of cell suspensions and serum were homogenous, demonstrating that these

15. A. S. Wiener, *Blood Groups and Transfusion, 2nd ed.* (Springfield: Charles C. Thomas, 1939).

16. R. A. Kilduffe and M. DeBakey, *The Blood Bank and the Technique and Therapeutics of Transfusions* (St. Louis: C. V. Mosby Co., 1942).

TABLE 1

Cold Agglutination Persisting at Room Temperature

Group AB Blood

	5° C	*Patient's Serum 28° C (Room Temperature)*	*37° C*
AB cells	++++	+	0
B cells	++++	+	0
A cells	++++	+	0
O cells	++++	+	0
O cells	++++	+	0
O cells	++++	+	0
Patient's cells	++++	+	0

Patient's Cells

	5° C	*Room Temperature 28° C*	*37° C*
O Serum	++++	+++	++
A Serum	++++	++++	++++
B Serum	++++	++++	++++
AB Serum	0	0	0

agglutinins were not active at body temperature. The reactions were reversible, clumps appearing again when the mixtures were cooled.

Considering this case as one possessing cold autoagglutinins, it was thought safe to transfuse an O blood since no AB blood was available. This was successfully accomplished without untoward reactions, after taking the precaution to warm the blood to 37° C before transfusion.

UNCLASSIFIED ISOAGGLUTININS

Irregular isoagglutinin reactions were observed in the blood of a patient suffering from nephritis and anemia. This blood was referred to the Blood Bank by Dr. J. A. Pons and Dr. E. O. Schlosser of the Presbyterian Hospital at San Juan. It was difficult to determine the patient's blood group because of strong autoagglutinins in the serum that apparently produced clumping of the cells before testing. To obviate this, the cells were washed twice in warm saline and observed under the microscope for evidence of clumping before setting up blood grouping tests. Using immune rabbit serum, the blood was finally classified as of group A, subgroup A_1, Type M.

TABLE 2
Titration of Unclassified Isoagglutinin
Blood Group A—Subgroup A_1

	Undiluted	*Patient's Serum*							
		½	¼	⅛	1/16	1/32	1/64	1/128	*Controls*
AB cells	++++	++++	++++	++++	++	0	0	0	0
A cells	++++	++	0	0	0	0	0	0	0
B cells	++++	++++	++++	++++	+++	++	0	0	0
O cells	++++	+	0	0	0	0	0	0	0
Patient's cells	++++	+	0	0	0	0	0	0	0

The serum in question clumped cells from all the blood groups as well as from the patient's. Thereafter, titrations of this serum were set up on five different occasions over a period of four months and always gave approximately similar titers. The results of these agglutination tests are shown in Table 2. As expected in an A blood, this serum agglutinated B and AB cells to a titer of 1 : 32 and 1 : 16, respectively, because of the normal isoagglutinins anti-B in A bloods. The homologous A cells, O cells, and the cells derived from the same individual were also agglutinated to a titer of 1 : 2.

In order to study the effect of temperature on these reactions, tests were set up at 5° C, room temperature, and at 37° C. The blood was kept at a temperature of approximately 37° C from the time it was collected to avoid the danger of binding the agglutinins to the red blood cells in case cold agglutinins were present. Pipettes, saline, and tubes were also kept at temperatures of 37° C. All cell suspensions were washed twice in saline and a control set up to eliminate the possibility of cell clumping previous to mixing with the serum. The results of these tests, shown in Table 3, revealed that these isoagglutinins were not affected by a change of temperature in contrast to the usual autoagglutinins that act best in the cold, occasionally at room temperature, but not at body temperature.

TABLE 3
Effect of Temperature on Agglutinin Reactions of Unclassified Isoagglutinin
(5° C—Room Temperature—and 37° C)

	Undiluted	*Patient's Serum*							
		½	¼	⅛	1/16	1/32	1/64	1/128	*Controls*
AB cells	++++	++++	++++	+++	++	0	0	0	0
A cells	++++	++	0	0	0	0	0	0	0
B cells	++++	++++	++++	++++	+++	++	0	0	0
O cells	++++	+	0	0	0	0	0	0	0
Patient's cells	++++	+	0	0	0	0	0	0	0

Absorption tests with A_1 and A_2 cells were undertaken to determine whether the atypical isoagglutinins would be absorbed by the homologous cells. The sediment of washed suspensions of A_1 and A_2 cells was mixed in a proportion of one part of cells to four parts of serum and left at room temperature for half an hour. They were then centrifuged and the supernatant serum tested for agglutinins (Table 4). The agglutinins were not bound to the A_1 and A_2 cells and only a nonspecific diminution in the titers against the cells of all blood groups was obtained. The possibility of this peculiar agglutinin reaction being related to the much discussed Rh factor was eliminated by the fact that the patient proved to be Rh positive when determined with guinea pig immune serum.

TABLE 4
Agglutination Tests of Unclassified Isoagglutinin after Absorption with A_1 and A_2 Cells
Patient's Serum Absorbed with A_1 Cells

	Undiluted	*Patient's Serum Absorbed with A_1 Cells*							
		½	¼	⅛	1/16	1/32	1/64	1/128	*Controls*
A_1 cells	++	0	0	0	0	0	0	0	0
A_2 cells	+	0	0	0	0	0	0	0	0
O cells	++	0	0	0	0	0	0	0	0
Patient's cells	++	0	0	0	0	0	0	0	0
B cells	++++	++++	+++	++	++	0	0	0	0
AB cells	++	++	++	+	+	0	0	0	0
		Patient's Serum Absorbed with A_2 Cells							
A_1 cells	+	0	0	0	0	0	0	0	0
A_2 cells	++	0	0	0	0	0	0	0	0
O cells	++	0	0	0	0	0	0	0	0
Patient's cells	++	0	0	0	0	0	0	0	0
B cells	++++	++++	++++	+++	+	0	0	0	0
AB cells	+++	+++	++	++	+	0	0	0	0

Recently Wiener[17] reported a case of hemolytic anemia in a sixteen months' old baby who died following a transfusion of incompatible blood. A strong autoagglutinin, active at body temperature, was demonstrated in this baby's blood, and there was speculation as to the part played by such autoagglutinin in the development of the acute hemolytic anemia and in the production of the transfusion reaction. Error in the grouping of this case was probably due to the presence of this powerful autoagglutinin in the baby's serum. In

17. A. S. Wiener, "Hemolytic Reactions. I. Diagnosis with Special Reference to the Method of Differential Agglutination," *Am.J.Clin.Path.*, 12:189, 1942.

his paper Wiener mentioned an unpublished case of Dr. Kracke's, describing an autoagglutinin active at body temperature. Dr. Kracke's patient also died despite transfusion therapy.

From Bellevue Hospital Reisner and Kalkstein[18] reported a case of autoagglutination in a forty-three year old Chinese, in whose blood the autoagglutinins were active at body temperature. Their patient belonged to group A, subgroup A_1, type MN, and his serum contained, in addition to the autoagglutinin, anti-O and anti-A_2 agglutinins apparently derived from a previous transfusion. The two transfusions given him were followed by rapid destruction of the transfused blood. In an attempt to eliminate the chief site of blood destruction, the enlarged spleen was removed, after which it was found that transfusions of A_1 blood were tolerated despite the persistence of strong autoagglutinins in the patient's serum. This case also terminated fatally on the ninth post-operative day, with profuse hemorrhages in the intestinal tract. Although no autohemolysins were demonstrated *in vitro*, it was evident that they were present *in vivo*.

A search through the literature has failed to disclose other cases of autoagglutinins, active at body temperature, other than the three already mentioned. In contrast to the ones already described, the case reported here has shown no evidence of spontaneous blood destruction. The patient has never been jaundiced nor has he ever received a blood transfusion; no autohemolysins have been demonstrated in his serum. Whether these autoagglutinins are active *in vivo* is questionable, at least in this case. Blood transfusion has been withheld from this patient as it is feared that the autoagglutinins may acquire autohemolysinic properties, if a transfusion be given. The patient has responded somewhat to hematinics and liver extract and it is felt that the risk involved in a transfusion is too great to warrant its administration.

SUMMARY

In a series of 1,016 cross-matching tests, four bloods were found to contain irregular isoagglutinins:

1. Anti-Rh agglutinins in the blood of a woman who had recently delivered an erythroblastic infant.

18. E. H. Reisner and M. Kalkstein, "Autohemolysinic Anemia with Autoagglutination: Improvement after Splenectomy," *Am.J.Med.Sc.*, 203:313, 1942.

2. An A_1 blood containing isoagglutinins active against A_2 and O bloods.

3. Cold agglutinins reacting at room temperature.

4. Unusual isoagglutinins in the blood of a chronic nephritic for whom transfusion was sought. These isoagglutinins were active against cells from all blood groups as well as against the patient's own cells, agglutination persisting at 37° C. In view of these findings, the transfusion was withheld. Review of the literature has shown that only three similar cases have been reported, all of which terminated fatally following blood transfusion.

ACKNOWLEDGMENT

The authors acknowledge herein the technical assistance of Miss Aida Torruella and Mrs. M. H. Costa Mandry, of the Blood Bank.