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NATURAL ANTI-M AGGLUTININS IN THE SERA OF MALE NEGROID TWIN INFANTS, WITH A COMPARISON OF DIFFERENT METHODS OF TREATING ANEMIA[1]

by

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The hereditary nature of red cell agglutinogens is well established. (1) With regard to antibodies, any that are present in the serum at birth have been acquired passively from the mother by placental transfer,[2] and when the individual later forms his own antibodies these apparently represent the result of immunization. This is true even of the " naturally " occurring anti-A and anti-B isoagglutinins. (3) However, the specificity of the antibodies is conditioned by the agglutinogens present in the red cells, since, for example, Rh_0-positive individuals cannot be sensitized against the Rh_0 factor and only Rh_0-negative individuals can be so sensitized. The suggestion has been made that the capacity to form antibodies in general is conditioned by the constitution of the individual. (4, 5, 6) In this report we propose to present some observations on like sexed twins, which are pertinent to this problem.

Treatment of anemia by transfusion is a common practice, although many types of anemia can be treated more conservatively and just as satisfactorily by oral medication with preparations containing iron, vitamin B_{12}, etc. There is a paucity of carefully controlled reports comparing various methods of treatment, so that in this paper we propose to compare the effectiveness of different methods of therapy in a pair of anemic like-sexed twins.

Case report

The patients are a pair of male Negroid twins who were born on January 26, 1951. They are their mother's sixth and seventh children and the result of her sixth pregnancy. The mother's antepartum course was uneventful and the delivery was normal and spontaneous after a 38 week gestation. Twin A weighed 3 lbs. 4 oz. at birth; twin B, 3 lbs. 6 oz. Both twins exhibited early retrolental fibroplasia and were placed on a course of ACTH therapy, starting at 2 weeks of age. The therapy was continued for a month and then the infants were discharged to be followed up in the well baby clinic.

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[2] Recently an exception to this rule has been encountered, namely, (2) a newborn infant with severe hemolytic anemia due to high-titered cold autoagglutinins present in his serum, but absent from the mother's serum.

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Table 1 - Comparison of clinical course of twin A and twin B

DATE (1951)	Feb. 21	July 20		July 27	July 30	Aug. 3	Aug. 7	Sept. 17		Oct. 12		Nov. 12
Twin B												
Hb. %[3]	63	37		29	29	38	34	47		59		58
RBC (mill.-cmm.)	—	3.76		2.6	2.65	2.83	2.64	3.06		3.25		4.53
WBC	14,800	6,800		7,700	7,750	9,050	8,250	7,550		9,500		7,900
Polys (Seg.	—	25		30	30	36	32	39		43		33
(Band	—	2		4	2	3	5	1		3		2
Lymph	—	61		64	62	60	58	57		49		57
Eos.	—	2		2	3	0	3	0		2		3
Monos.	—	10		0	3	1	2	3		3		5
Reticulocyres (%)	—	—		5½	4	10	7	2		—		4
Anti-M titer (units) at refrigerator temperature	—	—	Placed on therapy with ferrous sulphate by mouth	16	—	—	—	—	Placed on therapy with ferrous sulphate, vitamin B12 and folic acid, orally	—	Therapy with ferrous sulphate, vitamin B 12 and folic acid continued.	4
Twin A												
Hb (%)	59	30		67	68	63	—	56		58		58
RBC (mill.-cmm.)	2.99	3.51		3.88	4.05	4.15	—	3.65		3.24		4.24
WBC	19,600	6,500		8,050	7,500	8,500	—	9,750		8,250		8,600
Polys (Seg.	—	20		30	35	37	—	45		40		38
(Band	—	2		5	2	2	—	4		2		3
Lymphs	—	68		64	61	59	—	51		55		55
Eos	—	3		0	2	2	—	0		0		2
Monos.	—	7		1	0	0	—	0		3		2
Reticulocytes (%)	—	—		rare	0.5	0.5	—	0.5		—		rare
Differential agglutination test, percent donor's cells surviving	—	—	Transfusion 100 cc. packed RBC	—	100	—	75	15	Placed on ferrous sulphate	8	All therapy discontinued	0
Anti-M titer (units) at refrigerator temperature	—	—		1½	—	—	—	—		—		0

[3] 100% = 14 Gms-100 c.c.

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At the age of one month, while still in the hospital, the twins were observed to be pale, and blood counts showed twin A to have a hemoglobin concentration of 59 percent, twin B a hemoglobin concentration of 63 percent (cf. table 1). This was classified at the time as " anemia of prematurity ". The twins returned to the clinic on July 20, 1951 when 6 months of age, and it was observed that the infants' pallor had increased markedly. A blood count revealed the hemoglobin concentration for twin A to be only 30 percent, and for twin B, 37 percent (cf. table 1). Blood transfusion was requested, and while no difficulty was encountered in crossmatching twin A's blood, strong clumping was observed in the crossmatching of twin B's blood. Accordingly, twin A was given a transfusion of 100 c. c. of packed red cells, while twin B was placed on oral iron therapy pending further studies. At this point the case was called to the attention of the present author.

Grouping tests showed both twins to belong to group B. It was possible to verify the observation that in testson the well slide, twin B's serum strongly clumped the blood cells not only of group A, but also of groups O and B. Twin A's serum strongly clumped blood cells of group A, as expected, and there was also faint clumping of blood cells of other groups. Since both twins belong to type N, the possibility suggested itself that we were dealing with natural anti-M agglutinins. Therefore, a series of 10 bloods were tested and reading taken blind (cf. table 2), and the results showed that the antibody in twin B's serum which had caused the strong clumping in the crossmatching tests was indeed an anti-M agglutinin. Moreover, in tests at refrigerator temperature it was possible to demonstrate a weak anti-M agglutinin in twin A's serum as well. The anti-M agglutinins were " cold " agglutinins. While twin B's serum reacted also at room temperature, clumping did not occur at body temperature; the weaker anti-M agglutinin in twin A's serum was active at refrigerator temperature only.

Table 2 - Demonstration of anti-M agglutinins in the sera of twins

Serum of	Serum mixed with 2% blood suspension from 10 different persons: readings after one hour in refrigerator.									
	ON	BN	OM	BN	BN	OMN	ON	ON	OMN	OM
Twin A	—	—	++±	—	—	++±	—	—	++±	++±
Twin B	—	—	+±	—	—	+±	—	—	+±	+±

It was then decided to do a complete study on the family in order to determine whether other members of the family possessed anti-M agglutinins (cf. table 3). The mother and the two oldest children were found to belong to type N, but their sera did not contain demonstrable anti-M agglutinins (Incidentally, the family is of interest as an example of the mechanism of the heredity of the Rh-Hr types, in view of its large size and the unusual types of the parents).

Table 3 - Results of grouping tests on the family of the twins

Blood of	A-B-O group	M-N type	Rh-Hr type		Kell type	Duffy type
			Phenotype	Genotype		
Father	A_1B	MN	Rh_1rh	R^1R^o	kk	ff
Mother	O	N.ss	$rh'rh$	$r'r$	kk	—
1st child ♀	B	N.ss	Rh_o	R^or	kk	—
2nd child ♀	A_1	N	Rh_1rh	R^1r or R^or'	kk	ff
3rd child ♀	B	MN	Rh_o	R^or	kk	ff
4th child ♀	B	MN	Rh_1rh	R^1r or R^or'	kk	ff
5th child ♀	B	MN	Rh_1rh	R^1r or R^or'	kk	ff
Twin A ♂	B	N.ss	Rh_o	R^or	kk	ff
Twin B ♂	B	N.ss	Rh_o	R^or	kk	ff

An attempt was then made to account for the anemia, which the twins had already exhibited while still in the hospital. The fact that the mother belonged to group O and the twins to group B suggested that sensitization of the mother to agglutinogen B might have played a rôle. (7, 8, 9, 10) The alpha and beta antibodies in the mother's serum were therefore titrated and the results are shown in table 4. As can be seen the mother is strongly sensitized to the agglutinogen B indicating that this may indeed have been the primary cause of the twins' anemia, which perhaps was aggravated by a deficiency of iron-containing foods in their diet. On the other hand, there were no Rh antibodies in the mothers serum.

Table 4 - Titer of the isoantibodies in the sera of the mother and the twins

Serum of	Method of titration	Titer (units) for cells of group			
		0	A_1	A_2	B
Mother	Agglutination	0	200	75	400
	Plasma conglutination	0	400	100	240
	Acacia conglutination	0	480	300	2000
Twin A	Agglutination method	0	32	—	0
	Plasma conglutination	0	40	—	0
	Acacia conglutination	0	60	—	0
Twin B	Agglutination	0	8	—	
	Plasma conglutination	0	5	—	0
	Acacia conglutinatio	0	40	—	0

It was decided to take this opportunity to compare conservative methods with transfusion therapy for treating the anemia exhibited by the twins. Accordingly, twin B was placed on oral ferrous sulphate therapy, and blood counts were taken periodically on the twins. As shown in table 1, the hemoglobin concentration of twin B rose progressively while that of twin A gradually declined until after two months on September 17, 1951, the blood counts closely approximated one another.

Table 5 - Titration[4] of anti-M agglutinins in serum of twin B

Date specimen obtained[5]	Test cells	Dilutions of twin B's serum tested against untreated red cells							Dilutions of twin B's serum tested against enzyme-treated red cells[6]				
		Undiluted	1:2	1:4	1:8	1:16	1:32	1:64	Undiluted	1:2	1:4	1:8	1:16
August 3, 1951	OM	++±	++±	+++	++	++	+±	+	—	—	—	—	—
	OMN	++±	++±	+++	++	+±	—	—	—	—	—	—	—
	ON	—	—	—	—	—	—	—	—	—	—	—	—
November 13, 1951[7]	OM	++	+±	+	—	—	—	—	++	+±	±	—	—
	OMN	++	+±	—	—	—	—	—	++	++	+±	+	—
	ON	±	—	—	—	—	—	—	++	++	+±	—	—

[4] Titrations were carried out at refrigerator temperature.

[5] The specimen taken in August had been stored in the frozen state and was thawed out to be used in the experiment, so that the titrations could be carried out in parallel with the November 13th specimen.

[6] The enzyme used for treating the cells was ficin.

[7] The tests were repeated again four months later, while this article was inpress, and at that time the anti-M agglutinins were no longer demonstrable.

Therefore, twin A was also placed on oral ferrous sulphate therapy, while for comparison the treatment of twin B was changed to oral combined iron, vitamin B_{12}, and folic acid. A month later the blood counts of the twins were practically identical, and at this time iron therapy of twin A was discontinued while therapy was continued for twin B. As shown in the table the further progress of the twins was the same, so therapy was also discontinued for twin A in December.

During the period of observation, we studied the course of the titer of the anti-M agglutinins in the sera of the twins (cf. table 5). Anti-M agglutinins could not be demonstrated in any specimen of twin A's serum aside from the pretransfusion sample, and in this, as already mentioned, the titer was so low that the agglutinin was not detectable in the routine crossmatching tests. The titer of the anti-M agglutinin in twin B's serum gradually fell, and after four months the titer was only 4 units at refrigerator temperature, that is, only one-fourth the titer when first detected. Tests with enzyme-treated cells revealed that cold autoantibodies were being formed, while the anti-M agglutinins dropped in titer. Differential agglutination tests on twin A's blood showed that the rate of elimination of the donor's cells from the infant's body was approximately normal for his age, so that the weak anti-M cold agglutinins in his serum apparently did not interfere with the survival of the donor's cells, even though the latter belonged to type MN.

Table 6 - Comparison of the capacity of untreated and enzyme-treated red cells to absorb anti-M agglutinins

	Serum of twin B [8]	Anti-M fluid prepared from immune rabbit serum [9]
Titer (units) of unadsorbed serum	10	9
Titer after adsorption with untreated OM cells	0	0
Titer after adsorption with enzyme-treated OM cells	5½	11

As shown in table 5, the anti-M agglutinins in twin B's serum produced no clumping at all when the test cells were treated with proteolytic enzymes (In the experiments described here, the enzyme used was ficin). [10] This contrasts sharply with the behavior of A-B-O antibodies, Rh-Hr antibodies, and autoantibodies, which generally react in higher titer against enzyme-treated cells. 11 Adsorption

[8] Titrations at refrigerator temperature.
[9] Titrations at room temperature.
[10] Kindly provided by Merck and Co.

tests (table 6) on twin B's serum, as well as anti-M fluid prepared from immune rabbit serum, showed that enzyme-treated cells are incapable of adsorbing M antibodies, confirming previous reports. (12, 13, 14) If, as these investigators suggest, this is due to destruction of the agglutinogen M by the enzyme, this would indicate that the structure responsible for the specificity of the agglutinogen M is protein or polypeptide in nature.

Comment

The demonstration of the presence of the rare isoagglutinin, anti-M, in a pair of like-sexed twins lends further support to the theory that individuals exist who have a special constitutional capacity to form antibodies. Additional strong evidence supporting this idea is provided by instances of multiple sentitization to weak antigens. (15, 16, 17)

With regard to the source of the anti-M agglutinins in the twins' sera, one might consider that these are of " spontaneous " origin, as has been suggested for the isoagglutinins anti-A and anti-B. As has been pointed out elsewhere,[3] however, there is strong evidence indicating that the natural anti-A and anti-B agglutinins are not merely of spontaneous origin, but represent an heteroimmune response to A-like and B-like antigens in parasitic microörganisms and in food. If the anti-M agglutinins in the present case are of similar origin, as seems likely, this suggests that a search be made to determine what food in the diet or what microörganism or animal possesses M-like antigens.

The anti-M agglutinins in the twins described in this paper are the eighth and ninth examples of anti-M encountered by the present author among tens of thousands of human sera examined. In contrast, not a single example of anti-N has been encountered. Of the seven instances of M isoantibodies previously encountered, three were cold agglutinins and therefore presumably of heterogenetic origin. One of the cases (18) was similar to that described here, in that during cross matching tests prior to a blood transfusion the M agglutinins were discovered in the serum of a nine-month old baby with diarrhea and anemia. In one instance, (15) the cold anti-M agglutinin was associated with isoimmune Rh antibodies. In the remaining four instances the anti-M agglutinins were of isoimmune origin, and the titer was about the same at body and refrigerator temperature. In one of these latter instances, the M antibody was produced in response to a blood transfusion, (19) while in the other three instances, Rh-negative volunteers produced M as well as Rh antibodies after deliberate immunization in order to obtain Rh typing sera. (20) Transfusion of Rh-negative blood containing the M agglutinogen to one of the immunized donors caused an hemolytic reaction, (21) demonstrating again the greater danger of reactions from isoantibodies active at body temperature in contrast to cold agglutinins.

A similar situation exists regarding antibodies for the special agglutinogen present in group O blood. Almost all O antibodies that have been studied to date either occurred spontaneously in human sera, especially from individuals of

subgroup A_1B, or in animal sera, especially from cattle or eels, or were produced by immunization of goats with Shiga bacilli. Thus, these O antibodies are presumably of heterogenetic origin, and like the natural alpha and beta antibodies react best at low temperature. Rarely anti-O has occurred in human serum as a result of isoimmunization, such as in the individual studied by Henry (22) and the present author. (23) As is to be expected, the latter variety of anti-O isoagglutinins reacted with about the same titer at body temperature as at lower temperatures.

Comparison of the progress of the blood counts of the twins demonstrated that the ultimate result was the same in the twin treated conservatively by oral iron therapy, although, of course, the effect was immediate in the twin who received the blood transfusion. Since it has been found that transfusions are not entirely innocuous, and may give rise to isosensitization, and at times to unpleasant or even dangerous reactions, they should not be resorted to except when absolutely necessary. Certainly, in the present case the desired goal was attained just as satisfactorily and at less expense by using the more conservative therapy. The question arises whether these infants would not have done just as well on a complete diet with adequate quantities of meats and vegetables and without supplementary iron medication. Should we ever encounter another pair of twins with a similar anemia, we intend to treat one with oral iron medication, and, for comparison, keep the other on a full diet without any drugs or transfusion.

Summary

A pair of male negroid twin infants with natural anti-M agglutinins in their sera is described. It is pointed out that this observation lends further support to the thesis that the general capacity to form antibodies has a constitutional basis.

The twins had a severe anemia, apparently due in part to sensitization of their group O mother to the group B agglutinogen in their red cells, and in part of nutritional origin. One infant was treated by blood transfusion and the other was given oral medication containing iron. Both twins recovered and their blood counts became virtually identical at the end of a period of observation of three months.

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RIASSUNTO

L'articolo descrive una coppia di bambini gemelli negri che avevano nei loro sieri agglutinine naturali anti-M. L'A. sostiene che questa osservazione conforta la tesi che, in genere, la capacità di formare degli anticorpi ha una base costituzionale.

I gemelli presentavano una grave anemia in parte dovuta alla sensibilizzazione della loro madre di gruppo O rispetto all'agglutinogeno di gruppo B posseduto dai loro globuli rossi, e in parte di origine alimentare. Un bambino fu trattato con trasfusione di sangue mentre all'altro venne somministrato un medicamento orale contenente del ferro.

I due gemelli vennero ricuperati e il tasso dei loro globuli rossi divenne virtualmente identico al termine di un periodo di osservazione durato tre mesi.

RÉSUMÉ

L'article décrit une paire de jumeaux nègres, petits enfants, qui avaient agglutinins anti-M naturels dans leurs séra. L'auteur indique que cette observation prête de l'appui supplémentaire à la thèse que la capacité générale de former des anticorps a une base constitutionnelle.

Les jumeaux avaient un anémie sévère, apparemment occasionnée partiellement par la sensibilité de leur mère, qui avait le sang groupe O, à l'agglutinogen B dans leurs cellules rouges, et partiellement par les facteurs nutritives. Un des enfants a été traité par une transfusion de sang et à l'autre était donné de la médication orale qui contenait du fer.

Les deux jumeaux se sont récupérés et les comptes de leurs cellules rouges sont devenus virtuellement identiques au bout d'une période d'observation de trois mois.

ZUSAMMENFASSUNG

Dies ist die Beschreibung eines männlichen, negroiden Zwillingspaares mit natürlichen anti-M Agglutininen in ihren Seren. Sie zeigt, dass die Beobachtung zu einer weiteren Unterstützung der Theorie führt, nach welcher die allgemeine Fähigkeit Antikörpern zu bilden eine wohl unterstützte Grundlage hat.

Die Zwillinge hatten eine schwere Anämie, offenbar teilweise in Folge der Sensibilisierung ihrer, der Gruppe O angehörenden Mutter gegen die Gruppe B Agglutinogen in ihren roten Blutkörperchen und teilweise in Folge des Ursprunges ihrer Nahrung. Ein Säugling wurde mittels Bluttransfusion behandelt, während den Anderen Eisen enthaltende Medikamente eingeflösst wurden. Beide genasen und am Ende der drei monatlichen Beobachtungsperiode war das Blutbild Beider fast gleich.

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