ANTIGENS OF THE BLOOD GROUPS IN ONTOGENESIS

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The study of the antigenic differentiation of the cells of the human body is not only of theoretical interest, but also of great practical importance to the solution of problems in forensic medicine, to the elucidation of the pathogenesis of hemolytic disease of the newborn, the explanation of posttransfusional reactions, and the solution of the problem of tissue compatibility.

Reports in the literature of the transplantation of various tissues (in particular, bone marrow) taken from embryos are becoming more and more frequent [5, 11]. The success of these grafts is attributed to the lower activity of the embryonic antigens. Hence it has become necessary to make a careful study of both the time of formation of antigens in ontogenesis and the degree to which they are manifested. This is especially so because the information given in the literature regarding the time of appearance of the ABO, MN, P, and Rh antigens is completely contradictory, and the time of appearance in embryogenesis of other antigens – Fy^a, Kell-Cellano – is not known; only relatively few papers have been published on the subject of the study of the degree of activity of antigens in the embryo.

The group antigens of the ABO system are known to appear early in the human erythrocytes – at the end of the third month, i.e., at the 11th-12th weeks of embryonic life [2, 4, 7, 14, 15, and others]. Such workers place the time of group differentiation later – the 4th-5th months, and O. M. Zemtsova and A. A. Terekhova [3] later still – at $6\frac{1}{2}$ months of intrauterine life.

According to most writers, M and N antigens can be found in the erythrocytes of 9- and 11-week embryos [1, 4, 8, 15, 17].

So far as the Rhesus system is concerned, the information in the literature is based on isolated observations. Several writers [8, 9, 18] have found Rhesus antigen in the erythrocytes of 16-32-week embryos. V. A. Strukov claimed having found it in an embryo at the 8th-9th week of intrauterine development.

P antigen is found in embryos at the end of the 3rd and beginning of the 4th month [10, 13, 16]. Very little information is available on the time of formation of Duffy (Fy) antigen. Race and Sanger [16], for instance, detected it in one fetus at the 17th week of embryonic life. Cutbush and Mollison [9] found it in newborn infants. So far as O and Kell-Cellano antigens in embryos are concerned, we have found no reference to this subject in the literature.

It is thus clear from the foregoing that certain antigens, such as O, Kell-Cellano, and rh, have not been investigated during embryogenesis, and that studies of the time of formation of the Rh_0 and Fy antigens are incomplete.

We set out to make a comparative immunological study of the formation of the group antigens of the ABO, Rhesus, MN, Fy, Kell-Cellano, and P-p systems in the course of embryogenesis. It was also considered of interest to compare the time of formation of a series of antigens to the same object, namely erythrocytes taken from one embryo.

EXPERIMENTAL METHOD

Material for the investigation (human embryos) was obtained from maternity homes and gynecological hospitals in Moscow. We investigated the blood of 94 embryos aged between 10 and 28 weeks of intrauterine life. The investigations were conducted with the aid of the appropriate specific antisera: anti-A and anti-B (human isosera with natural antibodies); anti-M, anti-N, and anti-O (hetero-immune rabbit sera); anti-Rh_0, -rh', and rh" (iso-immune human sera with complete anti-bodies); anti-Fy^a and anti-Kell-Cellano (iso-immune human sera obtained from the Blood Donor Service Institute, New York, with incomplete antibodies).

Depending on the character of the serum, different methods were used to study the antigens. The A, B, O, M, and N antigens were investigated by the agglutination reaction on a flat surface, and the O antigen by the same method and also in tubes, followed by centrifugation by Schiff's method. The Rh_0, rh', and rh" antigens were investigated by the tube method. The result was recorded by the character of the pattern on the base of the tube after incubation of a 2% suspension of the test erythrocytes with the corresponding antiserum for 1 hour at 37°. The rh', Fy^a, and Kell-Cellano antigens were studied by means of the Coombs' test. The P antigen was investigated like the Rhesus factor in tubes, but the mixture of erythrocytes and anti-P serum was incubated in a refrigerator at 4° and not at 37°.

EXPERIMENTAL RESULTS

An idea of the distribution of the A, B, M, and N antigens in embryos may be obtained from Table 1, from which it is clear that the erythrocytes of all the embryos investigated showed differentiation within the limits of these systems. Ten-week embryos were no exception in this respect. It must be pointed out that the correlation between the different groups of antigens was the same as that observed in adults. Table 2 shows that the corresponding antigens can be found in the erythrocytes of 10-14-week embryos of intrauterine development. The relative proportions of the Fy^a (+) and Fy^a (-), Kell (+) and Kell (-), and Cellano (+) and Cellano (-) antigens in these embryos were also the same as in adults.

TABLE 1. Distribution of ABO and MN Antigens in Embryos of Different Ages

Age of embryos (in weeks)	No. of fetuses tested	ABO system				MN system		
		O	A	B	AB	M	N	MN
10-14	5	1	2	2	–	1	1	3
15-19	30	8	10	11	1	11	2	17
20-24	34	11	12	9	2	11	4	19
25-28	25	8	13	2	2	10	7	8
Total	94	28	37	24	5	33	14	47

TABLE 2. Distribution of $Duffy^a$, Kell, and Cellano Antigens in Embryos of Different Ages

Age of embryos (in weeks)	No. of fetuses tested	$Duffy^a$		Kell		Cellano	
		+	–	+	–	+	–
10-14	4	3	1	–	4	4	–
15-19	29	25	4	2	27	29	–
20-24	24	17	7	1	23	24	–
25-28	21	14	7	–	21	21	–
Total	78	59	19	3	75	78	–

TABLE 3. Distribution of Rh_0, rh', and rh" Antigens in Embryos of Different Ages

Age of embryos (in weeks)	No. of fetuses tested	Rh_0	$Rh'_0{}^{"}$	Rh'_0	$Rh"_0$	rh'	rh"	rh'	rh–
10-14	5	–	2	2	–	–	–	–	1
15-19	30	4	3	17	3	2	–	–	1
20-24	34	3	8	12	5	–	2	–	4
25-28	25	2	1	19	2	–	–	–	1
Total	94	9	14	50	10	2	2	–	7

It will be clear from Table 3 that Rhesus antigen Rh_0 and its variants rh' and rh" were clearly distinguished in embryos at the 10th week of embryonic life. It is interesting to note that of the 12 embryos investigated for the presence of rh' antigen, it was found in 6, and in 2 cases, moreover, it was shown to be present in embryos aged from 10 to 14 weeks.

A positive agglutination reaction with anti-O serum could be obtained in only 4 of the 28 embryos of Group O tested, whereas human adult erythrocytes always gave a well marked agglutination reaction with this serum. In this respect our results agree with those of Formaggio [12] and Watkins [19], who consider on the basis of their investigations of newborn infants that O antigen begins to be formed only after birth, and that the antigen reaches the adult level of activity only after 1 year.

So far as the P antigen is concerned, this was found in 13 of 60 embryos. The number of P-negative embryos, according to our results, exceeded the corresponding number of adults and children; moreover, in the embryos we observed a weaker hemagglutination reaction. Hence it may be considered that P antigen in embryos shows a lower activity than in adult erythrocytes.

Our investigations thus showed that A, B, M, N, Rhesus, Duffy, Kell, and Cellano antigens are formed in embryos at the stage of 10 weeks of intrauterine life. The distribution of these antigens in embryos does not differ from their distribution in adults. The exception to this rule is the P antigen, in relation to which a higher percentage of P-negative individuals was observed than in adults, indicating that the process of formation of this antigen in the embryos is incomplete.

The erythrocytes of embryos between the 10th and 28th weeks hardly react with anti-O serum, i.e., they are undifferentiated in respect of this antigen; its formation evidently takes place at a later period.

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