EXPERIMENTAL BIOLOGY

PROBLEM OF THE IMMUNOBIOLOGICAL INTERRELATIONS OF MOTHER AND HUMAN FETUS

COMMUNICATION III DETERMINATION OF GROUP ANTIGENIC SUBSTANCES IN FETAL MEMBRANES

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(Received for publication on January 9, 1956. Submitted by Acting Member of the Acad. Med. Sci. USSR, N. N. Zhukov-Verezhnikov)

The conflicting nature of the data on the determination of the group antigenic differentiation of fetal membranes [3, 5, 6, 7] has necessitated further study on this question.

The part of the chorion forming the placental tissues cannot fail to play a decisive role in the immuno-functional interrelations of mother and fetus and therefore we considered that it must possess one or another group specificity.

Moreover, the previous findings obtained by us [2] establishing that the group antigens of the amniotic fluid do not originate in the blood of the mother or fetus but are probably produced by the amniotic tissue brought us to investigate this tissue with the object of establishing its group adherence.

EXPERIMENTAL METHODS

The tissue investigated (amnion or chorion) was washed with running tap water until the visible blood admixture was removed, and then washed three times with physiological saline. The washed tissue was boiled in physiological saline for 10 minutes in order to soften it, which simplified subsequent treatment of the tissue in preparing the aqueous suspension. After cooling, the tissue was cut in small pieces with scissors, then pulverized in a porcelain mortar. The ground up mass after being washed 3-4 times and after the addition of a further portion of physiological saline was decanted into agglutination tubes and centrifuged for 10 minutes (2000-2500 revs/min). The centrifugate was removed and the tissue residue was subjected to examination. Subsequently we examined both the centrifugate and the tissue residue and the centrifugate alone.

The residue of pulverized tissue obtained after centrifugation was diluted with physiological saline to give a 50% suspension. 0.25 ml of this suspension was poured into each of two agglutination tubes - to one tube was added 0.25 ml of normal isoagglutinating serum α, and to the other 0.25 ml of serum β. The mixture was carefully shaken and kept for 30 minutes at room temperature, then from the first tube, two drops of the liquid were drawn off and transferred successively to a number of tubes each containing two drops of physiological saline to give various dilutions: 1:2, 1:4, 1:8, 1:16, 1:32; from the latter, the fifth tube, the surplus two drops of fluid were removed. The two drops remaining in the second test tube (with serum β) were also successively transferred through a number of tubes containing physiological saline and diluted to the same degree, from 1:2 to 1:32. Then to each tube was added one drop of 3% suspension of normal human erythrocytes: in the first series — group A, in the second — group B, in order to determine the degree of combination of the isoagglutinins of the standard sera by the antigens of the investigated tissues. Absence of an agglutination

reaction or reduction in the degree of agglutination in comparison with the control allowed us to judge the presence of group substances in the fetal membranes. We investigated a total of 77 treated samples of amnion and 67 of chorion tissue residues.

EXPERIMENTAL RESULTS

The results of the investigation of the group antigens in the fetal membranes in the treated tissue residues are presented in Table 1.

TABLE 1

Determination of Group Antigens in Treated Tissue Residues of Fetal Membranes.

Material investigated	Number of investigated samples	Group A-B antigens not found	Group antigens found		
			A	B	AB
Amnion	77	10	22	18	27
Chorion	67	17	15	15	20

As is clear from Table 1 the cells of the amnion and chorion contained one or other group antigen. Thus, of 77 samples of amnion the presence of A-antigens in 22 samples was established, B - in 18, AB in 27; in 10 amnion samples neither A nor B antigens were found. Of 67 chorion samples examined antigens A were detected in 15, B antigens in 15 and AB in 20; 17 samples of chorion did not give combinations of either α or β agglutinins and consequently were free of group substances.

In order to solve the question as to whom the group substances found in the amnion and chorion belong – mother or fetus - we compared the findings with the blood groups of the confined woman and the newborn. It was established that in the majority of cases the group specificity of the amnion cells corresponds to the blood group of the child and and chorion specificity to the blood group of the mother.

However in a series of cases in the investigated residual cells, group antigens, absent both from the maternal blood and the blood of the child, were detected or, on the contrary, the group antigens contained in the maternal blood or in the fetal blood were not discerned.

After obtaining such a result in which, on the one hand, the response was nonspecific and, on the other, negative, we assumed that this result was probably due to the exceptionally careful washing of the investigated tissues, which might have led to the loss of a definite number of group antigens contained in them*.

Then after investigating simultaneously both the residual cells and the saline extract of them, we became convinced that the really significant part of the group substances was in the solution used for washing (in the extract). For this reason subsequent determination of the group adherence of the fetal membranes was undertaken by us only in the saline tissue extract. Preliminary treatment of the membranes was conducted as in the investigation of the residual cells, but instead of a 50% suspension of residual cells the supernatant liquid was used in an absorption test for isoantibodies. Otherwise the investigation was performed by the above described method.

The findings on determination of the group substances in the saline extracts from the amnion and chorion tissues are given in Table 2.

It is clear from Table 2 that of 44 amnion samples tested no group antigens were found in 7, the presence of antigens A was established in 18, B in 14, and antigens AB were detected in 5 samples. Upon comparison of

* The possibility of group substances passing from the cells of the human organs to the physiological saline used for washing them was demonstrated in 1933 by L. A. Shvartsman and N. N. Zhukov-Verezhnikov [4].

these data with the blood groups of the confined women and newborn it was established that in all cases the group adherence of the amnion corresponded to the blood group of the child.

Of 42 investigated samples of chorion no group antigens were found in 8; the presence of antigens A was established in 18; B in 10; and 6 samples of chorion contained antigens AB; here it was revealed that in the majority of cases (39 out of 42 studied) the group adherence of the chorion corresponded to the blood group of the mother. In 3 cases the group homology of the chorion cells and the blood of the confined woman was not established and in all these cases group antigens A, contained in the maternal blood were not detected in the chorion. We suppose the reason for this was that antigens A in the chorion might have been in the form of Sub-group A_1 substances which, as is known, are not always detectable. Another explanation also cannot be precluded, namely that as the chorion upon being washed free from the blood is subjected to more thorough treatment than amnion, some of the group antigens contained in it might have been lost.

TABLE 2

Determination of Group Antigens in Saline Extracts of Amnion and Chorion Tissues (Taking Into Account the Blood Groups of Confined Women and Newborn)

Material Investigated	Number of samples investigated	Group A-B antigens not found	Group Antigens found			Group Homology With Blood established	
			A	B	AB	newborn	women in confinement
						in number of cases and in %	
Amnion	44	7	18	14	5	44 (100%)	–
Chorion	42	8	18	10	6	–	39 (92,8%)

Thus, our findings suggest that the fetal membrane tissues in the same way as other tissues of the human organism are differentiated group-wise.

The failure of other authors [3,5] who have studied the amnion and chorion tissues without finding in them group antigens is probably the result of errors in method. The negative results obtained by Oettingen and Witebsky [5] Chernikover and Zemtsova (3) may be ascribed to the fact that they investigated not saline but alcoholic tissue extracts whereas, as is known, the group antigens being polysaccharides, are not soluble in alcohol.

Of great interest is the fact that the membranes, in cases of different group adherence of the fetus and mother, inherit different antigens : chorion—antigens of the maternal blood and amnion— antigens of the fetal blood.

This fact requires suitable immuno-physiological clarification.

The positive solution of the question of the group differentiation of amnion and chorion refutes the view of Oettingen and Witebsky regarding the placenta as a neutral organ between mother and fetus, allegedly defending them in case of adherence to different blood groups.

We support the view expressed in the literature that where the group adherence of fetus and mother is different the fundamental barrier function is performed by the placenta and possibly by the fetal waters, however, the mechanism of mutual defense of mother and fetus in these cases is apparently not the neutrality of the placental tissues, but, on the contrary, results from the presence in them of corresponding group antigens.

There are grounds for assuming that the fall in titer noted earlier by us [1] of the corresponding isoantibodies in the retroplacental blood of confined women which occurs with different group adherence of mother and fetus is to be explained by the combination of these antibodies with the group antigens of the amnion (and possibly by the antigens of the fetal waters); the fall in isoantibody titer in the umbilical blood noted in these cases may be due to combination with the group antigens of the chorion identical with the antigens of the maternal blood.

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