SEROLOGICAL STUDIES IN EXPERIMENTAL FETAL ERYTHROBLASTOSIS

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In investigations which we carried out earlier [1, 2, 4] it was shown that the etiology, pathogenesis and clinical picture of hemolytic disease of newborn infants and experimental fetal erythroblastosis in rats are very similar.

In the present paper the results of serological studies which we carried out during an investigation of experimental fetal erythroblastosis* are given. We were primarily interested in the problem of the connection between the high titer of the anti-B_1-antibodies in the blood of pregnant females and the degree of hemolytic disease in their progeny.

METHODS

The blood sera of females (before mating, repeatedly during pregnancy, 24 h and 10 days after birth) and of the newborn rats was periodically studied for the development of anti-B_1-antibodies and the determination of their titer. The determination of the titer of the total anti-B_1-antibodies in the blood sera of the females and their progeny, and also the identification of the erythrocytes in the blood of the young rats, was carried out by the hemagglutination reaction according to the usual method.

Anti-B_1-antisera used for identification of the blood of the young rats was obtained by immunizing chinchilla rabbit with erythrocytes from the blood of adult B_1-positive homozygotic rats. Before setting up the reaction these sera were adsorbed with the erythrocytes of the blood of B_1-negative rats [10].

Coombs' direct assay with the erythrocytes of the young rats was carried out with antiglobulin serum obtained by immunizing rabbits with rat serum. Antiglobulin serum with a precipitin titer of 1 : 8000-1 : 16,000 after absorption (for the removal of hemagglutinins) with erythrocytes of adult B_1-positive rats was used in the work.

Incomplete anti-B_1-antibodies in the blood serum of the females and young rats was titered with erythrocytes treated with trypsin [9].

At the same time, the presence of B_1-antigen in the tissues and fluids of the experimental animals was determined. For this the specific absorption reaction of anti-B_1-antibodies from isoimmune sera with water-salt extracts of the tissues being studied or with 50% suspensions of cell sediments of these same tissues was used [3, 6].

Work on the experimental study of hemolytic disease of the newborn included three series of experiments: I, control, series of experiments carried out on animals of related line (♀ and ♂ B_1-1-♀; ♀ and ♂ B_1+/+), series II and III—on animals incompatible as to B_1-antigen (♀ B_1-1-X♂ B_1+/+). In addition, in some of the cases observations were made only during comparison of immunologically incompatible parent couples; in others the immunological conflict between the female and offspring was additionally strengthened by active and passive immunization of the females [1, 2, 4].

*The identification of the blood of the rats used in our studies was carried out by Frenzl et al. [10]; the antigen which they found in the erythrocytes of black rats was conditionally designated by the symbol B_1. This designation has no relationship to the denotation of the blood antigens of the ABO system in man and animals.

Results of Experiments on the Reproduction of Experimental Fetal Erythroblastosis in Rats

Series of experiments	Immunization of the female with B_1-antigen	Number of young rats studied	Titer of complete anti-B_1-antibodies		Clinical result of pregnancy	
			in the blood serum of females	in the blood serum of newborn rats	hemolytic disease of offspring	Newborn rats without symptoms of disease
I. ♀B_1—/—×♂B_1—/—	—	37	0	0	—	37
♀B_1+/+ ♂B_1+/+	+	25	1:64—1:128—1:256 (1:128)	(1:16)	—	25
II. ♀B_1—/—×♂B_1+/+	—	63	1:8 1:64— (1:16)	0—1:2 (1:2)	—	63
III. ♀B_1—/—×♂B_1+/+	+	188	1:128 1:2048 (1:256)	0—1:64 (1:10)	188	—

Note. Average value of antibody titers is given in parentheses.

RESULTS

In the control group, in spite of immunization of the females with B_1-positive erythrocytes and the presence of anti-B_1-antibodies in their blood serum, the progeny developed normally and there was not noted in the newborn rats any kind of clinical, hematological and pathological-anatomical signs of disease. Anti-B_1-antibodies, formed in the female organism, appeared also in the blood serum of the newborn rats, but did not cause hemolytic disease, since all the young rats were B_1-negative. Similar results were obtained in rabbits [11].

In the series of experiments carried out on animals with an incompatible blood group, in 3 out of 6 females, as a result of pregnancy, sensitization occurred to the B_1-antigen of the male (progeny). In the blood serum of these females 6-12 hours after birth there were found complete anti-B_1-antibodies in a titer of 1 :8, 1 :16 and 1 :64.

In the blood serum of the rats born of the latter female (having an antibody titer of 1 :64) complete anti-B_1-antibodies in a titer of 1 :2 were found. However, in this series of experiments pregnancy in the animals also developed normally and all the offspring (6 litters with a total number of 63 rats) did not have symptoms of the disease. This data agrees with clinical observations and also with the results of studies carried out by a number of authors on other species of animals [9, 12].

Evidently, during pregnancy the mother is immunized by the antigens of the offspring, but the degree of sensitization is insufficient to cause disease of the offspring.

Under experimental conditions, hemolytic disease of the newborn can be caused only by increase additional immunization of the female. The results of the studies which are presented in the table show that hemolytic disease of the offspring arises in those cases in which immunological incompatibility between the female and offspring is increased by additional immunization of the female [1, 2, 4, 8, 10].

Clinical observations of hemolytic disease of the newborn in humans during Rh-incompatible pregnancy indicate the presence of a link between the degree of sensitization of the mother (repeated pregnancies, transfusions of incompatible blood) and the expression of hemolytic disease of the offspring [5, 13].

It is necessary to note the paradoxical (on first glance) fact that in the blood of the most heavily diseased young rats anti-B_1-antibodies do not appear or are observed in lower titers than in the young rats in the control series of the experiments. There is reason to suggest that during fetal erythroblastosis the antibodies which pass through the placenta into the embryos are combined in large amounts with the cells of the organs of the young rats, aggravating their disease. This data agrees with clinical observations [7].

The studies which we made show that in B_1-positive rats and new born rats the B_1-antigen is contained not only in the erythrocytes but also in the fixed cells of organs and tissues, and also in the fetal membranes. We succeeded in establishing the presence of B_1-antigen in the serum of the amniotic fluid and saliva of B_1-positive rats. In this respect the B_1-antigen is very similar to the Rh-antigen [6].

In the young rats of the experimental group the B_1-antigen is not present in the chorion, and this agrees with clinical observations shown in the group differentiation of fetal membranes in humans [3].

In our experiments a connection was noted between the high titer of anti-B_1-antibodies in the blood serum of females and the discovery of these antibodies in the blood serum of young rats; the titer of 1 :64, evidently, is the threshold concentration of antibodies in females below which it is not possible for them to pass through the placenta into the blood of the fetus.

A high titer of antibodies in the blood of the female and the absence of antibodies in the blood of the offspring are poor prognostic signs. This experimental data cannot be discounted during serological analysis of appropriate clinical cases. In the hemagglutination reaction between the erythrocytes from the blood of diseased young rats and the blood serum of females containing anti-B_1-antigens in high titer, in half of the cases the result was unfavorable. Evidently, in these cases the antigenic receptors of the erythrocytes of the blood of young rats are blocked by incomplete antibodies which can much more easily pass through the placenta than complete antibodies. In some animals the presence of incomplete anti-B_1-antibodies was established in the blood serum of isoimmunized females in a titer of 1 :2048-1 :8192 and in the serum of young rats in a titer of 1 :64-1 :256 respectively. Coombs' direct assay with erythrocytes of the blood of newborn rats both in the experimental and in the control group was positive in all cases. However, 24-30 h after birth the reaction with erythrocytes of the control young rats became negative, while with the erythrocytes of diseased young rats the reaction was still positive for 2-3 days. It is evident that Coombs' direct assay in the first days after birth of the rats cannot serve as a specific diagnostic test.

According to our observations, antibodies in the blood serum of some females in a sufficiently high titer (up to 1 :128) could be found even 30-40 days after birth. In the blood of newborn rats complete antibodies in a number of cases were found for eight days after birth, while on the 8th day the titer was 1 :2.

For clarification of the possibility that antibodies are transferred from the mother to the offspring by means of lactation we studied the stomach contents of young rats (6-10 h after birth) from 3 experimental females. It was found that complete anti-B_1-antibodies present in the blood of the females in sufficiently high titer (1 :512) could be found both in the blood serum and in the stomach contents of newborn rats. However, the condition of the rats after birth was not aggravated. From this it can be presumed that the transfer of antibodies by lactation evidently does not play a significant role in the pathogenesis of fetal erythroblastosis in rats.

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