THE IMMUNO-ADAPTIVE STAGE IN RATS IN RELATION TO NORMAL AND HETEROLOGOUS CELLS

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According to data in the literature [1, 2, 3, 5], it is possible to induce in rats an immunological adaptation in relation to heterologous antigens (of mouse, rabbits or man) both during embryogenesis and in the early postembryonic period. We do not yet know for how long in the early postembryonic period the newborn rats preserve their powers of immunological adaptation. There are reports [4] that in the duck the stage of adaptation towards heterologous antigens lasts from the 6th to the 13th day after hatching.

In the present investigation we studied the immuno-adaptive stage in rats in relation to normal heterologous cells.

EXPERIMENTAL METHOD

Newborn rats, aged under 24 hours and 2, 3, 4, 5, 10, 15 and 20 days, were given a single subcutaneous injection of 0.2 ml of a 40% suspension of thrice-washed rabbits' red cells in the dorsal region. After 2 months the rats were immunized intraperitoneally with three doses of rabbits' red cells in the form of a 40% suspension (the first two doses of 0.5 ml each at intervals of 24 hours, and the third dose of 0.5-0.8 ml on the 4th-7th day after the second dose).

The second group of rats, also aged under 24 hours, received subcutaneous injections of 0.1 ml of citrated rabbits' blood. As in the preceding group, the rats were immunized at the same age with three intraperitoneal injections (each of 1 ml of citrated rabbits' blood at intervals of 2 weeks between doses).

The serum used for the determination of the agglutinins and their titers was taken from all the animals on the 9th-10th day after injection of the last immunizing dose. Before immunization, the serum of the experimental and control animals was investigated for the persence of natural heteroagglutinins against rabbits' red cells.

The rats were reimmunized on attaining sexual maturity and a weight of over 150 g, the methods of immuization, taking the sera and determining the agglutinin titer being exactly the same as at the first immunization.

In precisely the same way a 40% suspension of washed red cells from white mice was injected. The mouse red cells were injected into rats in a dose of 0.1-0.2 ml within 24 hours of birth and then at the age of 36 hours, and 5, 9 and 15 days. On attaining a weight of 50 g these animals were immunized intraperitoneally with three doses, each of 1 ml of citrated blood, at intervals of 3 days.

The animals which were injected with the suspension when less than 24 hours old were reimmunized 4 weeks

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General Scheme of the Results of Immunological Adaptation of Rats in Relation to Heterologous Red Cells

Age of animal and mode of injection of antigen	Mouse red cells	Homogenized mouse tissues	Rabbits' red cells	Human red cells
Single injection:				
to embryos 3-4 days before birth	+	+	+	±
to newborn rats	+	+	+	–
24 hours after birth	+	+	+	–
36 hours after birth	–	–	–	
5 days after birth	–	–	–	
9 days after birth	Immunity	Immunity	Immunity	
15 days after birth	"	"	Immunity	
20 days after birth			"	
Second injection:				
to embryos 3 days before birth				
to newborn rats 1-5 days after birth				+
to newborn rats on the 1st-16th day of life				+

Legend: + production of statistically significant tolerance; ± production of tolerance not statistically significant; – no tolerance produced.

after the last dose of the first immunization by the method indicated. In these cases too, serum for the estimation of the immune agglutinins was taken on the 10th day after the last immunizing dose.

EXPERIMENTAL RESULTS

The effect of injection of mouse red cells in the postembryonic period was investigated in 19 newborn rats. A statistically significant decrease was found in the formation of immune agglutinins by comparison with 15 control rats both after primary immunization and reimmunization.

The decrease in the formation of immune heteroagglutinins against rabbits' red cells was observed in 22 experimental animals which received injections of antigen when under 24 hours of age. On comparison of the mean titers with those of a control group of 24young rats of the same age, taken in the majority of cases from the same litters as the experimental animals, a statistically significant difference was found between the mean titers in respect to a decrease in their ability to form antibodies. Before reimmunization no convincing difference could be found between the mean titers in the experimental and control animals. The same thing was observed after reimmunization. Immunological adaptation to the rabbits' red cells which was created in the newborn rats was weaker than that induced in embryos.

In agreement with the results obtained in the preceding investigations, we were unable to demonstrate the presence of any natural heteroagglutinins in the rats. Injection of mouse red cells to young rats 36 hours after birth and on the 5th day of life did not induce adaptation, but after injections of mouse red cells on the 9th day of life, in some experimental immunized animals higher titers of immune heteroagglutinins were found than in the control animals.

We obtained values for experimental and control rats which were injected with rabbits' red cells on the 2nd-20th day of life. The agglutinin titers before and after immunization showed that injection of cellular antigen on the 2nd-5th day of life had no essential effect on the formation of agglutinins in adult rats. This is a sort of zero period. Beginning on the 10th day of extrauterine life, injection of antigens now caused the appearance of immune agglutinins in some rats before immunization, and the appearance of higher titers than in the control animals – after immunization.

Our experiments showed that the stage of immunological adaptivity in rats in relation to mouse and rabbits' red cells when giving in a constant dose (0.1-0.2 ml of a 40% suspension) ended on the first day after birth. By using a phylogenetically more distant antigen, for example human red cells, injection of the same volume of suspension to animals of the same age, however, did not now cause adaptation. In order to produce this degree of adaptation in these cases a far greater dose of antigen was required [3].

Between the 2nd and 5th day of life the volume of antigen used caused neither adaptation nor an immune reaction; starting on the 9th-10th day it caused antibody formation in certain animals.

The table gives in schematic form an assessment of our findings concerning the immunological adaptation of rats in relation to heterologous antigens.

The conclusion of the stage of immunological adaptivity must always be determined in respect to a given antigen and to a definite dose.

The characteristics and the duration of the so-called zero period, in which neither adaptation nor immunity is observed, remain uncertain. From the results of our previous experiments on the study of the immunological adaptation of rats to human red cells, it follows that by using a particular antigen in doses not reaching a certain threshold value, the "zero period" may be shown as that period of development of the animals in which a higher dose will still induce adaptation.

Thus the duration of the so-called zero period is also relative, i.e., it always depends on the particular antigen and the particular dose. This also follows from experiments studying homologous adaptation, which showed that it was possible to produce tolerance in rats until the 14th day of extrauterine life [6], i.e., when we obtained for the same dose of heterologous antigens either the zero period or a period of commencing antibody formation.

SUMMARY

The immuno-adaptive period in rats in relation to a single administration of mouse and rabbits' red cells ends during first day after birth. Administration of these red cells from the 2nd to the 5th day after birth causes neither immunological adaptation nor antibody formation. Beginning from the 9th or 10th day of life a single injection of the same doses causes antibody formation in some animals.

LITERATURE CITED

[1] W. Bolag, Experientia, 1955, 11, 227.

[2] J. Grozdanovic, Cs. biologie, 1956, 5, 300.

[3] J. Grozdanovic, Cs. biologie, 1957, 6. 87.

[4] V. Haskova, Cs. biologie, 1957, 6. 81.

[5] A. Puza and J. Molnar, Cs. biologie, 1956, 5. 304.

[6] M. F. Woodruff and L. O. Simpson, Brit. J. Exp. Path., 1955, v. 36. 494.