STUDY OF REPRODUCTIVE FUNCTION IN SENSITIZED FEMALE RATS

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UDC 612.63.017.3

Reproductive function was studied in female rats immunized with homologous splenic antigens. Primary injection of tissue antigens in a total dose of 88 mg protein induced the formation of antibodies against spermatozoa in the females and led to disturbance of reproductive function (sterility, abortions, stillbirths, or high neonatal mortality). Subsequent injection of tissue antigens into these females in doses more than 20 times higher than the primary dose (up to 2000 mg protein) abolished their manifestations of immunological conflict and restored the normal reproductive function. It is postulated that in this case the therapeutic effect is due to the development of an immunological paralysis of the Felton type.

Insufficient attention has so far been paid to the study of ways of overcoming the immunological conflict between mother and fetus arising during pregnancy and producing various clinical manifestations (stillbirth, spontaneous abortion,hemolytic disease of the newborn). Only isolated reports of the induction of tolerance in order to overcome a conflict arising during pregnancy can be found in the literature [2]. In experiments on rats it has been shown that injection of tissue antigens of August rats into Wistar females induces a state of sensitization in the recipients, whereas injection of the same antigens during drug-induced sleep leads to the creation of tolerance. More than half the sensitized females became sterile; among the tolerant animals one rat in thirty was sterile.

Other workers [4] used $B_1^-/_-$ female rates and $B_1^+/_+$ male rats in their experiments. On different days after birth, the females were injected with B_1-positive blood in order to induce tolerance against B_1-antigen. When the females had reached sexual maturity they were then immunized with B_1-positive blood and mated with males of blood group $B_1^+/_+$. A sharp decrease in specific antibody formation was observed in these females. The optimal conditions for obtaining an effect of tolerance were: repeated (up to 3 times) injections of the antigen; beginning of immunization on the 1st day of life. Primary injection of the antigen on the 5th day induced only partial tolerance, while on the 10th day no effect of tolerance was produced. Females in which tolerance was induced on the 1st day of life had clinically healthy offsping. If tolerance was induced on the 5th day, many of the offspring suffered from hemolytic disease of the newborn, and if induced on the 10th day all the fetuses showed evidence of hemolytic disease.

In the author's investigations tolerance was induced in adult female rats previously sensitized. These experimental conditions were thus more similar to clinical conditions in which it is most commonly necessary to suppress or reduce as much as possible the degree of sensitization in pregnant women. An earlier paper [3] described a model of the immunological conflict between mother and fetus in rats developed by the author.

In the investigation described below, this model was used to induce acquired tolerance. The method suggested by Efimov [1] was used in the experiments. Its essential feature is that the donor's antigens are administered while the general activity of the recipient's immunogenetic system is depressed. Amobarbital was used as a weak immunodepressor.

Department of Biology, I. P. Pavlov Ryazan' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 11, pp. 69-71, November, 1972. Original article submitted December 20, 1971.

TABLE 1. Outcome of Pregnancy and Parturition in Experimental and Control Rats

Group of animals	Character of expt.	No. of females	Reprod. function and outcome of pregnancy, parturition						
			secondary sterility	Intrauter. absorp. of fetuses	stillbirth	No. of fems. giving birth to nonviable offspr.	Death of females during parturition	Death of fems. from other causes	Normal parturization, viable fetuses
1st	Intact animal (control)	40	—	2	1	—	1	1	35
2nd	Primary immunization with tissue antigens in a total dose of up to 88 mg protein	60	24	5	8	13	4	3	3
3rd	Secondary injection of antigen in dose of 2000 mg protein								
4th	In conjunction with sorbitol	13	2	—	—	—	—	—	11
	Without amobarbital	30	—	—	—	2	—	1	27

EXPERIMENTAL

Observations were made on 143 female Wistar and noninbred albino rats weighing 180-250 g with a normal estrous cycle. To rule out the possibility of primary sterility, rats which had had a normal pregnancy terminating in the birth of healthy and viable offspring were used. The experimental group consisted of 43 animals, and the two control groups accounted for the other 100 rats. Group 1 (control) was composed of 60 females preliminarily immunized [3] and remaining under observation for 14 months; group 2 (control) consisted of 40 healthy rats. All the experimental females had previously been immunized and remained sterile for over 40 days or had other clinical manifestations of immunological conflict (stillbirth, hemolytic disease of the newborn).

The supernatant from homogenized homologous testicles was used as the antigenic material. Organs were taken from 20 animals sacrificed at the same time, homogenized with physiological saline (in the ratio of 1:4), and thoroughly homogenized for 10 min. The resulting homogenate was kept in a sterile receiver at 4°C. The protein concentration in the supernatant was determined refractometrically after 24 h.

The animals of group 1 (13 females) were injected with antigen + amobarbital. The supernatant from the homogenate was injected subcutaneously into the anterior abdominal wall in a dose of 76-90 mg protein in 5 ml fluid every other day for 1.5 months. Each rat was injected with 1800-2000 mg specific protein. Amobarbital was injected subcutaneously in 0.5% solution in a dose of 0.5 mg/100 g body weight. The females of group 2 (30 rats) received injections of the same doses of antigen but without amobarbital.

After the end of induction of tolerance, the females in a state of estrus were mated with males. The stage of the estrous cycle was determined and a diagnosis of fertilization and the initial stages of pregnancy made from examination of vaginal smears. The titer of antibodies against spermatozoa in the blood serum of the experimental and control rats was determined by the CFT. Sensitization of the newly born rats was determined by the direct Coombs' test in the modification described in [5].

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. In most (91%) of the experimental females fertilization and conception took place immediately after mating. No evidence of microspermagglutination was found in the vaginal mucus. In every case pregnancy ended in the birth of a healthy viable offspring. The direct Coombs' test with the blood serum of the young rats was negative.

In the group of rats receiving primary immunization (group 1 – control) the offspring of 26 females suffered from hemolytic disease of the newborn; in these cases the Coombs' test was positive. Microspermagglutination was found in smears of vaginal mucus from females remaining sterile longer than 60 days. No such phenomena were observed in any of the healthy animals of control group 2.

The CFT, carried out with the blood serum of the experimental and control animals, showed that the titer of antibodies against spermatozoa in the tolerant rats was sharply reduced (1:1) or no antibodies

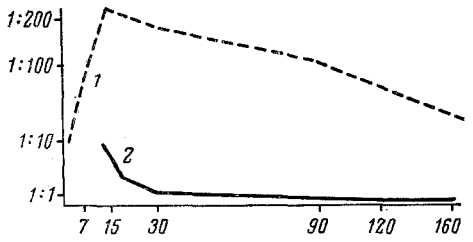

Fig. 1. Dynamics of titer of antibodies against spermatozoa in female rats with different states of their immunogenetic system. Abscissa – days after formation of immunity and tolerance; ordinate – titer of antibodies against spermatozoa. 1) Immunized females; 2) tolerant females.

whatever appeared. The character of the relationship between the antibody titer and the state of the immune system in the female determined by these experiments is shown in Fig. 1. The relatively high level of the antibody titer in the immunized rats and its sharp decrease in the rats with tolerance of their immunogenetic system demonstrate the important role of humoral factors during the formation of immunity and tolerance.

No significant difference was found between them when the results of the experimental groups 1 and 2 were compared. Probably the powerful antigenic stimulation of the female's immunogenetic system was not only specific, but also general. The action of the weak immunodepressant (amobarbital) therefore had no significant effect on the induction of tolerance.

These results are in agreement with others [6] indicating high and low zones of tolerance formation to an antigen. Primary injection of tissue antigens in a total dose of 88 mg protein induced the formation of antibodies against spermatozoa in the females and caused a disturbance of reproductive function (sterility, stillbirth, high neonatal mortality). The subsequent injection of tissue antigens into the same females in a dose more than 20 times higher than the first (up to 2000 mg protein) abolished their manifestations of immunological conflict and restored normal reproductive function. Presumably in this case the therapeutic effect was due to the development of immunological paralysis of the Felton type, a form of immunological tolerance, in the females.

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