ROSETTE FORMATION BY HUMAN, GUINEA PIG, AND RAT LYMPHOCYTES WITH SPERMATOZOA

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Cells forming rosettes with homologous or heterologous spermatozoa were found in the thymus, spleen, and bone marrow of sexually mature guinea pigs and of 14-30-week-old human fetuses, and also in the peripheral blood of men suffering from sterility. On the development of autoimmune orchitis after measured trauma to the testis in rats or after immunization of guinea pigs with testicular tissue homogenate mixed with Freund's complete adjuvant, cells forming rosettes with spermatozoa were found to appear in the spleen and thymus of the rats, and their number in the lymphoid organs of the guinea pigs increased. These procedures had no effect on the number of cells forming rosettes with sheep red blood cells in the lymphoid organs of rats and guinea pigs. The possible use of this newly discovered ability of human and animal lymphocytes to form spontaneous and immune rosettes with spermatozoa as a means of assessing the degree of differentiation of lymphocytes and of their sensitization to spermatozoal antigens in cases of disturbance of spermatogenesis of autoimmune nature is discussed.

KEY WORDS: *rosette formation test; spermatozoa; lymphocytes.*

The rosette formation test, revealing specific receptors on the surface of T and B lymphocytes and giving evidence of the degree of their differentiation or sensitization, has been widely applied in immunological research [2, 3, 5-9, 11-13]. After heterogeneity of antibodies had been established, a leading place in theoretical and experimental immunology was occupied by attempts to solve the problem of the heterogeneity of lymphocytes, both at the various stages of their differentiation and with respect to their ability to recognize antigens [5]. The rosette formation method is one of the most promising in investigations of this type.

Data indicating that human, rat, and guinea pig lymphocytes form spontaneous and immune rosettes with homologous and heterologous spermatozoa are presented below.

EXPERIMENTAL METHOD

The thymus, spleen, and bone marrow of 11 human fetuses at 14-30 weeks of development, of 22 sexually mature Wistar rats weighing 290-340 g, and of 18 sexually mature guinea pigs weighing 518-570 g, and also peripheral blood from 20 men aged from 25 to 40 years with childless marriages were investigated for cells forming rosettes (RFC) with homologous and heterologous spermatozoa. Besides intact animals, guinea pigs receiving a single injection of 1 ml homologous testicular tissue homogenate mixed with Freund's complete adjuvant (1:1) into the footpad also were used. Tests also were carried out for cells forming rosettes with spermatozoa on the lymphoid organs of rats in which orchitis was induced by measured trauma to the testis (puncture of the organ with a needle with an external diameter of 3 mm). The autoimmune nature of the posttraumatic orchitis was shown by the writers previously [4].

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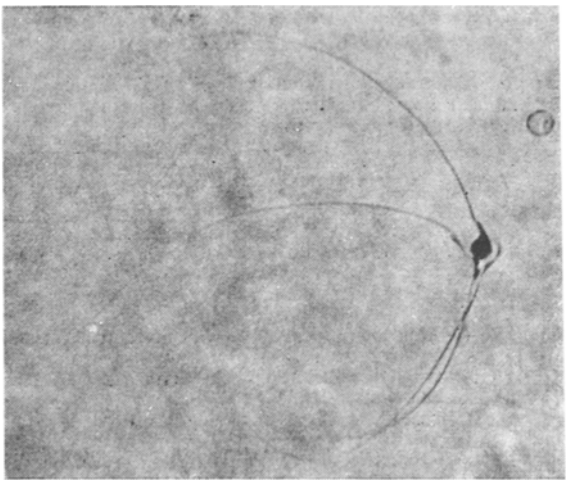

Fig. 1. Rosette formation by splenic lymphocyte from rat subjected to measured injury to the testis with homologous spermatozoa. Hematoxylin–eosin, 500×.

TABLE 1. Rosette Formation by Cells of Human Fetal Lymphoid Organs and Human Peripheral Blood

Indicator cells	% of RFC			
	fetal lymphoid organs			human peripheral blood
	thymus	spleen	bone marrow	
Sheep red cells	63 (35–80) $n=1$	0,61 (0–2) $n=4$	0,05 (0–0,5) $n=10$	28 (8–58) $n=18$
Rat spermatozoa	4,1 (0–8) $n=10$	0,83 (0–2) $n=4$	0,9 (0–10) $n=6$	2,5 (0–12) $n=9$
Human spermatozoa	—	—	—	1,6 (0–13) $n=18$

Legend. Here and in Table 2: limits of variation shown in parentheses.

The animals were killed 7, 14, and 21 days after the experimental procedure. Their testes were weighed and fixed in Carnoy's fluid, and paraffin sections 5 μ thick were stained with hematoxylin–eosin.

Cell suspensions from lymphoid organs were prepared by means of the liquid disintegrator suggested by Arkhipenko and Chuich [1], suspensions of bone marrow cells by perfusion of the femoral marrow of human fetuses or animals with cold medium No. 199, and lymphocytes were obtained from heparinized human peripheral blood after centrifugation for 30 min at 1800 rpm in a Ficoll-Verografin (Triosil) gradient. The resulting cell suspensions were washed twice with medium No. 199 cooled to 4°C, and the relative proportion of living cells was determined in percent by staining with 0.4% solution of trypan blue. Suspensions containing 90-95% of living cells were used and their concentration was adjusted to $4 \cdot 10^6$ cells in 1 ml.

Suspensions of spermatozoa were obtained from the epididymis of sexually mature rats or guinea pigs and also from fresh ejaculates from men donors, washed three times in medium No. 199. The concentration of spermatozoa was adjusted to $8 \cdot 10^6$/ml.

To determine the number of RFC, equal volumes (0.3 ml of each) of suspensions of lymphocytes and spermatozoa were mixed, incubated at 37°C for 15 min, centrifuged for 5 min at 1000 rpm, and kept in a refrigerator (4°C) for 90 min. After careful resuspension, 200 lymphocytes were counted under the microscope in a Goryaev's counting chamber and the relative percentage of RFC lymphocytes to which three or more spermatozoa were attached was determined.

TABLE 2. Effect of Measured Trauma to the Testis in Rats and Immunization of Guinea Pigs with Homologous Testicular Tissue Homogenate Mixed with Freund's Complete Adjuvant on Rosette Formation by Cells from Lymphoid Organs with Homologous and Heterologous Spermatozoa

Group of animals	Indicator cells	% of RFC in lymphoid organs		
		thymus	spleen	bone marrow
Intact guinea pigs	Guinea pig spermatozoa	8,0 $n=1$	4,7 (1,0—9,0) $n=6$	0,9 (0—3,0) $n=7$
	Rat spermatozoa	2,0 $n=1$	1,0 (0—2,0) $n=2$	0,5 (0—1,0) $n=2$
Guinea pigs 14 days after immunization with homologous testicular tissue homogenate	Guinea pig spermatozoa	19,0 $n=1$	17,0 (6—35,0) $n=11$	6,2 (2,0—15) $n=9$
	Rat spermatozoa	2,9 (1,4—4,5) $n=2$	2,0 (0—4,0) $n=7$	6,1 (4,0—11,0) $n=6$
Intact rats	Rat spermatozoa	0 $n=6$	0 $n=6$	0,1 (0—0,5) $n=5$
	Sheep red cells	0 $n=6$	1,5 (0—3) $n=6$	0,12 (0—0,5) $n=6$
Rats 7 days after measured injury to testis	Rat spermatozoa	0,78 (0—2,5) $n=4$	3,5 (1—6) $n=4$	2,6 (1—4) $n=4$
	Sheep red cells	0 $n=4$	0,5 (0—1) $n=4$	1,5 (0—4) $n=3$

The suspensions of lymphocytes also were investigated in the rosette formation test with sheep red blood cells by the same method as with the spermatozoa. A suspension of thrice-washed sheep red cells containing $8 \cdot 10^7$ cells/ml was used.

EXPERIMENTAL RESULTS

In most cases of rosette formation with spermatozoa, adhesion of the heads of the spermatozoa to the surface of the lymphocytes was observed and only these rosettes were counted in the results (Fig. 1). Adhesion of spermatozoa to lymphocytes by their tails also was observed.

Thymocytes from 14-30-week-old human fetuses were found to form spontaneous rosettes with rat spermatozoa in the same way as with sheep red cells. However, the number of cells forming rosettes with sheep red cells was much greater than the number forming rosettes fetuses, starting with the 20th week of development, formed relatively few spontaneous rosettes both with sheep red cells and with rat spermatozoa. In 10 of the 18 blood samples from sterile men that were tested, cells forming rosettes with human spermatozoa, and in five of nine specimens, with rat spermatozoa, were found (Table 1).

In intact rats rosette formation by lymphocytes from the thymus and spleen with homologous spermatozoa was never once observed, but in two of the five animals tested such rosettes were formed with bone marrow cells. Meanwhile, spleen and bone marrow cells of intact rats formed rosettes with sheep red cells (Table 2).

A decrease in the weight of the injured organ (512-1001 mg) compared with the weight of the intact testis (1398-1650 mg) was observed 1, 2, and 3 weeks after measured injury to the testis, diffuse aspermatogenesis developed in the injured testis, and this was accompanied by infiltration of the interstitial tissue by plasma cells and lymphocytes in the early stages after the operation. Cells forming rosettes with homologous spermatozoa appeared for the first time 1 week after injury to the testis in the spleen and thymus of the experimental rats, and the number of these cells in the bone marrow increased (Table 2). At all subsequent times of the investigation cells forming rosettes with spermatozoa were found in the lymphoid organs of the injured rats: Their number varied from 0.75 to 1 in the spleen, from 0.8 to 2.5 in the thymus, and from 0.25 to 1 in the bone marrow. Meanwhile, the operation had no effect on rosette formation by lymphocytes from the thymus, spleen, and bone marrow with sheep red cells, for their number in the injured rats was no greater than in the intact animals.

Lymphocytes of the spleen, bone marrow, and thymus of sexually mature guinea pigs, without any additional treatment, were capable of forming rosettes with homologous and heterologous spermatozoa. Immunization of guinea pigs with testicular tissue homogenate mixed with Freund's complete adjuvant led to the development, after 2 weeks, of autoimmune orchitis

with a considerable increase in the number of cells in the thymus, spleen, and bone marrow forming rosettes with spermatozoa (Table 2).

The results of this investigation thus indicate that human, guinea pig, and rat lymphocytes form rosettes with homologous and heterologous spermatozoa. Immunization of the animals with spermatozoal antigens or measured injury to the testis cause the development of autoimmune orchitis and the appearance of cells forming rosettes with spermatozoa in the lymphoid organs of rats, or an increase in their number in guinea pigs, but do not affect the number of cells forming rosettes with sheep red cells.

In 1974, Rivenzon et al. [9] reported the formation of reversible rosettes by human thymocytes and rat peripheral blood lymphocytes with mouse Leydig cells. These workers did not observe rosette formation by these types of lymphocytes of intact rats with Sertoli cells, spermatogenic epithelial cells, or spermatozoa. In 1975 a further paper was published [14] on rosette formation by rat lymphocytes with autologous large testicular cells. Unfortunately, these authors did not identify precisely with which testicular cells — Leydig or Sertoli cells, or spermatocytes —the lymphocytes reacted in their experiments. To sum up the data in the literature it can be concluded that as yet no evidence has been published that human and animal lymphocytes form rosettes with spermatozoa.

The results of the present investigation are interesting from two points of view. First, they show that cells of another type, namely spermatozoa, can be used to study the surface receptors of lymphocytes, an important fact with respect to the discovery of the rules governing their differentiation. Second, the rosette formation reaction with spermatozoa can be used in clinical and experimental practice as a test for determining the degree of sensitization of lymphocytes to spermatozoal antigens in cases of disturbance of spermatogenesis of autoimmune nature and of immunological sterility.

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