ORGAN ANTIGENS IN THE SPERMATOZOA OF MAN

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In our preceding investigations using heteroimmune sera, it was established that human spermatozoa contain species-specific antigens, common to the erythrocytes, [9] and spermatozoa from a person with blood group AB were segregated into human spermatozoa of blood group A and of group B by means of agglutination with immunized rabbit antiserum of spermatozoa of a donor with blood group A [7, 9, 10].

In this work we undertook to study the question of whether spermatozoa contain organ antigens under conditions of human isoimmunization.*

PROCEDURE

Isoimmune antispermatozoa serum was obtained by immunization of one of us (R. P. Popivanov) with the isoantigenic blood formula: $0\alpha\beta$, Ms−, CcDEe, C^W, K^-, with the spermatozoa of a donor with the blood group $A\beta$, Rh^+. Directly after the ejaculation of the sperm, it was washed four times with physiological solution, suspended in 1 ml of the same solution with the addition of 20,000 units of penicillin, and after 1 h, was injected deep under the skin of the lower third of the dorsal surface of the shoulder, using $7\text{-}12 \times 10^6$ living spermatozoa in each injection. A total of six injections were administered: December 4, December 10, December 17, December 23, December 25, 1963 and January 4, 1964. Samples of immunized serum were taken before immunization, then four days after the third injection, eight days after the fifth injection and then 9, 25, and 70 days after the sixth injection. The serum was investigated for the presence of complement fixing antibodies (RSK at 37° in a system of 2.5 ml), spermoagglutinins (in a test tube and on a slide), and also in gelatin according to the method of Kibrik, spermoprecipitins (reactions of ring precipitation and precipitation according to the method of Ouchterlony), and group agglutinins to the erythrocytes (in the test tube and on slides).

The setup of RSK was accomplished in the following way: in a system of 15 test tubes with three controls (one without antigen, the second without antiserum, and the third without complement) 0.5 ml of antigen of lyophilized A-spermatozoa, diluted in physiological solution (1:200), was titrated. To each test tube 0.5 ml of 1 : 30 diluted immunized human serum was added (having at this time an anti-A-spermatozoa titer of 1 :64), 0.5 ml of the complement, and 1 ml of the hemolytic system.

The following cells, organs, and human biological fluids were used as antigens: spermatozoa and erythrocytes of persons of blood groups A and B; sperm plasma of persons of blood groups A, B, and 0; brain, thyroid gland, lungs, heart, liver, spleen and kidney; in addition, the brain, lungs, heart, liver, spleen, and kidney of the rabbit were used.

The antispermatozoa serum (with a titer 1 :32) used in part of the investigations was obtained in 1961 by immunization or rabbits with human spermatozoa of blood group A_1.

Certain technical details of the conduction of the corresponding reactions were reported in our preceding works [1, 7, 9, 10].

*The work was reported at a scientific conference at the Institute of Experimental Biology, Academy of Sciences, USSR, in Moscow, February 28, 1964.

Titer of Complement-Fixing Antibodies of Human Blood Serum, Type 0

No.	Human antigen (1-12) and rabbit antigen (13-18)	Time of investigation after immunization by human spermatozoa of blood group A					Rabbit antiserum to human spermatozoa blood group A
		before immunization	Four days after third injection	Eight days after fifth injection	Nine days after sixth injection	25 and 70 days after sixth injection	
1	Spermatozoa A	0	1:16(+)	1:32(+++), 1:64(++)	1:128(+++)	0	1:32(+++)
2	Spermatozoa B	0			1:256(+++), 1:512(+)	0	1:32(+++)
3	Sperm plasma A	0			1:128(+++), 1:512(+)	0	1:8(+++), 1:16(+)
4	Sperm plasma B	0			1:256(+++), 1:512(+++)	0	1:8(+++), 1:16(+)
5	Sperm plasma 0	0			1:128(+++), 1:256(++)	0	
6	Brain	0	1:16(++), 1:32(+)	1:32(+++), 1:64(+)	1:128(+++), 1:256(++)	0	1:4(++), 1:8(+)
7	Thyroid gland	0			1:256(+++)	0	
8	Lungs	0	1:32(++)	1:32(+++), 1:64(+)	1:128(+++)	0	1:4(+++), 1:8(++)
9	Heart	0	1:16(++), 1:32(+)	1:32(+++), 1:64(+)	1:32(+++), 1:64(++)	0	
10	Liver	0	0	1:16(+++), 1:64(+)	1:64(+++), 1:128(+)	0	1:2(+++)
11	Spleen	0	1:16(+++), 1:32(++)	1:16(+++), 1:32(++)	1:64(+++), 1:128(++)	0	1:8(+++)
12	Kidney	0	0	1:8(++)	1:128(+++)	0	1:8(+++)
13	Brain	0			1:128(+++)	0	
14	Lungs	0			1:64(+++), 1:128(++)	0	
15	Heart	0			1:128(+++)	0	
16	Liver	0			1:128(+++)	0	
17	Spleen	0			1:64(+++), 1:128(++)	0	
18	Kidney	0			1:128(+++)	0	

RESULTS OF THE INVESTIGATIONS

From the table it is evident that before immunization, the persons of group 0 did not contain any iso- or heteroantibodies in the serum. On the fourth day after the third injection, the beginning of the immunization process was successfully detected, clearly pronounced in relation to the spleen, lungs, and heat; more weakly pronounced in relation to the brain; this process was absent in regard to the liver and kidneys.

On the eighth day after the fifth injection, the titer of antibodies to the antigens increased 2-4-fold; antibodies to the liver and kidney appeared.

In further investigations using RSK, it was found that the serum collected at the same period contained not only antispermatozoa and antiorgan antibodies, but also an antigen, which stimulated an isoimmunization process. In the setup of the RSK, complete restraint of hemolysis was established in all the test tubes, with the exception of the second control (without antiserum). When the experiment was conducted with seven test tubes and three controls, i.e., with extreme dilution of the antigen 7×10^{-6} (practically pure physiological solution), the same results were obtained. Hence, it may be assumed that the investigated serum sample contained not only the antibodies determined, but also an A-spermatozoid antigen. The latter, in all probability, was present in the serum in the state of a weak complex or existed independently.

The following experiment was conducted to elucidate this question. In the setup of the RSK with the same isoimmune serum, instead of the antigen (A-spermatozoa), 0.5 ml of physiological solution was introduced into each test tube.

It was found that up to a 1 :32 dilution of the serum, inhibition of hemolysis also occurs without the antigen (i.e., only in the presence of the antiserum and the complement), which is evidence of the presence of a substance in the serum possessing antigenic properties.

These investigations confirm that on the eighth day after the fifth injection, the serum of an immunized person contained the A-spermoantibodies, with a titer of 1 :64, and the A-spermoantigen with a titer of 1 :32.

On the ninth day after the sixth injection, the titer of the A-spermoantibodies was equal to 1:28, with a considerably more intense restraint of hemolysis in the RSK. Moreover, an increase in the antibody titer with respect to other antigens was observed (see Table). In the serum samples collected on the 25th and 70th days after the sixth injection, no antibodies for the antigens used were detected.

In order to verify whether the specificity of the antibodies produced in immunization with A-spermatozoa is limited only to the species affiliation, or whether it is a matter of polyorganic antibodies, the specificity of which exceeds the limits of species, supplementary experiments were conducted using RSK in which the following systems were used: 1) the serum of an immunized person and antibodies from rabbit organs (brain, heart, liver, lungs, spleen, and kidney); 2) rabbit antiserum to human A-spermatozoa and antigens of human organs. It was established that human anti-A-spermatozoa serum fixes the abovementioned rabbit organ antigens in almost the same titers as human antigens (see table).

On the other hand, rabbit antispermatozoa serum (with a titer of 1 :32 with respect to A-spermatozoa) fixes antigens from human spleen, kidneys, and lungs in a titer of 1 :8 (+++), brain in a titer of 1 :4 (++), liver in a titer of 1 :2 (+++), and does not combine with antigens from the heart.

The results of other investigations, not cited in the table, may be summarized in the following way.

Before immunization, the titer of α-agglutinins in the serum of an investigated person of group 0 was equal 1 :32, while that of the β-agglutinins was 1 :64. On the fourth day after the third injection, an increase in the titer of α- and β-agglutinins to 1 :128 was noted. On the eighth day after the fifth injection and on the ninth day after the sixth injection, the titer of α- and β-agglutinins was equal to 1 :64. The same titers of α- and β-agglutinins were noted on the 25th and 70th days after the sixth injection. In not one of the serum samples before or after immunization were spermoagglutinins and spermoprecipitans detected.

The appearance of organ antibodies in the serum of a person of group 0, immunized by human group A spermatozoa, permits us to assume that in addition to the species [8, 9] and group [2, 3, 4, 6, 13] antigens, the spermatozoa also contains antigens of the seven organs that we used, and in all probability, of all the other organs as

well. This means that the organs of a given species of animal or man are genetically represented in the spermatozoa, at least with respect to the most important, basic antigens.*

In iso- or heteroimmunization, organic antigens of the spermatozoa may induce the development of the corresponding antibodies, which also react with the antigens of the corresponding organs. A confirmation of this should be the positive result in conducting the opposite antigen-antibody reaction, i.e., when the antibodies against the antigens of each organ would react (in RSK experiments) only with the spermatozoa and would entirely or almost entirely fail to react with the other organs (depending on the antigenic closeness created during evolution).

Such a concept corresponds to the data of Lewis [5], who revealed the presence of antigenic relationships between the testicular tissue and the brain, as well as by Weil et al. [15], who, using RSK, found that the antisera of rabbits and roosters for human spermatozoa contain antibodies to human kidneys, liver, spleen, and testicular tissue in a 1 :10 titer.

As for the fact of the presence of an antigen in the serum of an immunized person, it may be assumed that in this initial phase of the isoimmunization process, the antigen and antibody may exist separately. An analogous case was described by one of the authors under conditions of autoimmunization [1, 14].

It remains unclear why in our experiment the immunization of a person of group 0 with human A-spermatozoa does not induce the formation of spermoagglutinins, together with the complement fixing antibodies. The data that we have at our disposal are still insufficient for a definite conclusion. In all probability, the organ antigens that we detected belong to the class of autoantibodies, belonging to the same category as the autospermoagglutinins, detected by certain authors.

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*In our previous investigations of the nature and interrelationships of the species-specific antigens (agglutinogens) of human spermatozoa and erythrocytes, we established that the existence of complete antigenic identity between the "general," species antigens of human spermatozoa and erythrocytes cannot be assumed. We assumed that the antigens characterizing the species, detected in human spermatozoa, do not remain unchanged after fertilization, during embryogenesis and the following stages of ontogenesis until the formation of the adult organism; they retain their basic content and structure, but in the cells and tissues (for example, in the erythrocytes, we already find them somewhat modified, having lost certain properties or acquired new ones. In accord with this, we consider it essential to exclude the possibility of finding "primarily laid" energetic properties and the ratios detected in the cells of the body and biological fluids of the adult human organism, in the germ cells (in this case, in the spermatozoa).