HETEROGENEIC ANTIGENS OF *Escherichia coli* SIMILAR TO ANTIGENS OF HUMAN SPERMATOZOA

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In recent years the problem of heterogeneic "mimicking" bacterial antigens, cross-reacting with similar antigens of human cells and tissues, has engaged the attention increasingly of investigators in connection with the discovery of their role in the development of specific forms of pathology in certain infectious and noninfectious diseases [3, 4]. On the basis of the discovery of natural sperm antibodies in the blood and the presence of antigens in bacteria similar to various human antigens [2, 6, 8], it was suggested that heterogeneic antigens similar with antigens of the microflora may be present in human spermatozoa [5].

The object of this investigation was to study the antigenic composition of various strains of *Escherichia coli* and human spermatozoa.

EXPERIMENTAL METHODS

Modifications of the microprecipitation test [1] and the test of cytotoxic action of cellular antigens on immune lymphocytes [6] were used. Considering that soluble heterogeneic antigens can be isolated from spermatozoa and bacteria, they were treated with the detergent Triton X-100 by the method described previously [7].

Strains of *E. coli* of three serotypes — 08, 09, and 086 — were grown on protein-free medium to eliminate the influence of components of nutrient media [8]. To 1 ml of bacterial residue 5 ml of 1% Triton X-100 solution made up in 0.01 M Tris-HCl buffer, pH 7.2, was added. Treatment with this solution continued for 1-10 days in glass flasks with the aid of magnetic mixers at room temperature (16 to 22°C). Every day material was taken from the mixture for investigation by the double immunodiffusion method. A set of rabbit antisera obtained against human spermatozoa (blood groups A, B, and O) and against the tested strains of *E. coli*, was used in the experiments. Freshly ejaculated sperm from healthy subjects of groups B, O, and A, the spermal plasma isolated from them, and also spermatozoa, washed with physiological saline, as well as bacteria, were used in immunodiffusion tests and tests to study the cytotoxic action (CTA) of cellular antigens on immune lymphocytes of Sp-D rats. Immune lymphocytes were obtained from the spleens of animals 2 weeks after sensitization with the antigens described above. The suspensions of immune and intact lymphocytes were washed with Hanks' solution and added in a concentration of 10^7 cells/ml to the residues of bacteria (10^{10}) and spermatozoa (10^8). After centrifugation for 5 min at 1000 rpm, the tubes containing the mixtures were exposed for 1 h at 37°C (the super rapid method), after which 9 ml of Hanks' solution containing 1% trypan blue solution was added, the mixture was shaken, and the percentage of dead cells was counted in a Goryaev chamber.

EXPERIMENTAL RESULTS

Some of the results of immunodiffusion analysis are given in Fig. 1. Material of *E. coli* 08 and 09 cells, treated with detergent, contained several antigens, one of which (the

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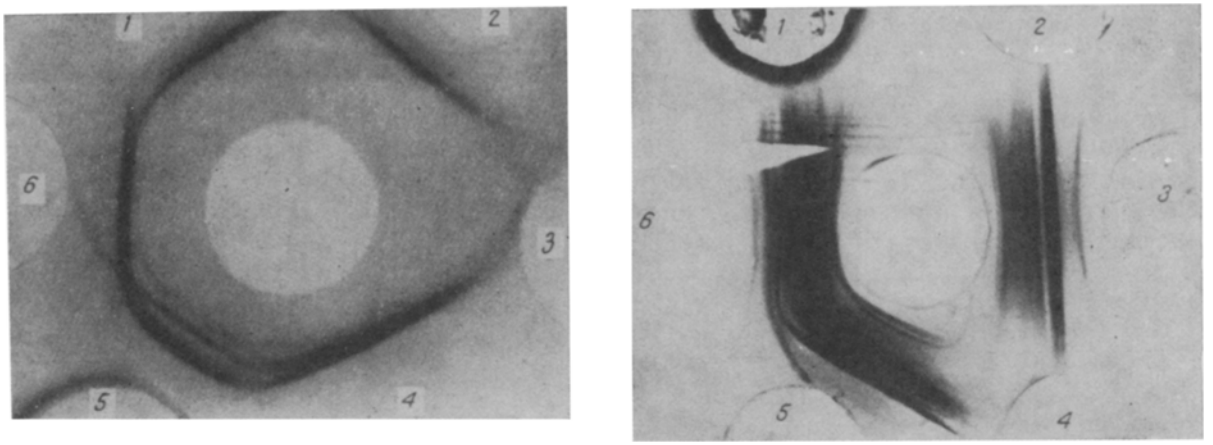

Fig. 1. Results of microdiffusion test in gel. a: Central well contains rabbit antiserum against *E. coli* 08, peripheral wells contain strains *E. coli* 08 (1) and 09 (2), treated with Triton X-100 for 3 days; human group A (3) and 0 (6) spermatozoa, intact strains *E. coli* 08 (4) and 09 (5); b: central well contains rabbit antiserum against human group 0 spermatozoa, peripheral wells contain strains *E. coli* 08 (1) and 09 (2), treated with Triton X-100 for 8 days, human group A (3), B (5), and 0 (6) spermatozoa, intact strain *E. coli* 08 (4).

outer precipitation band) was common with antigens of human spermatozoa of groups 0 and A, whereas the other (the middle dense precipitation band) was partially common with antigens of human spermatozoa of group 0 (Fig. 1a). One of the antigens of human spermatozoa of groups 0 and A (consisting of a thin precipitation band, located nearer to the wells), was similar to antigens of *E. coli* (Fig. 1b). The results of the other variants of the immunodiffusion experiments were identical and showed that at least one or two soluble antigens of *E. coli* serotypes 08 and 09 are similar to antigens of human spermatozoa. These antigens were found more clearly after treatment of the material for 3-8 days with Triton X-100, evidence that they are present in the deeper part of the cell membranes.

The presence of antigenic similarity between human spermatozoa and *E. coli* strains 08 and 086 was confirmed in crossed experiments with interaction between residues of spermatozoa and bacteria, on the one hand, and lymphocytes of rats immunized correspondingly with human group 0 spermatozoa and *E. coli* (intact lymphocytes of rats of the same Sp-D line in the control). The results of experiments with sedimentation of human groups 0, A, and B spermatozoa were identical and showed that whereas with intact lymphocytes the percentage of dead cells did not exceed 30, with immune lymphocytes against spermatozoa it exceeded 70-80, and with lymphocytes immune to *E. coli* it exceeded 40-50. In the *E. coli* precipitation experiments, in the control the percentage of dead lymphocytes varied from 20 to 40, whereas in the experiment, after contact with lymphocytes immune to *E. coli* it reached 60-70, and with lymphocytes immune to human spermatozoa it exceeded 40-60. These data were in full agreement with the results of immunodiffusion analysis undertaken previously.

E. coli cells of serotypes 08, 09, and 086 thus contain heterogeneous antigens similar to the cellular antigens of human spermatozoa and represented mainly on the surface of bacteria and spermatozoa. These antigens cannot be classed with the heterogeneous antigens similar to group antigens of the ABO system, for the former could not be detected in the chosen strains by the method of absorption of specific sera against A, B, and 0 antigens. The possibility evidently cannot be ruled out that these antigens may belong to the mannans of bacterial cell walls, which have been shown to be similar to the cellular carbohydrates of sperm [9]. The discovery of heterogeneous antigens, similar to the specific antigens of spermatozoa, in microorganisms not only helps to elucidate the mechanisms of development of sterility on the basis of crossed immunologic reactions, but also opens the way for the practical use of harmless bacterial "spermovaccines," essential for fertility control in man and animals.

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STUDY OF MECHANISM OF INHIBITION OF THE IMMUNE RESPONSE IN PARABIONTS OF DIFFERENT AGES

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When animals of different ages are joined together in parabiosis for a long time with common circulation and joint cell metabolism, the immune response of the younger partner is inhibited considerably whereas the stimulating effect of the younger partner on the older is small [1]. Other investigations [7, 10, 11] of this problem have shown that the older partner or its tissues are carriers of information accelerating ontogenetic development, which "age" the younger partner on close contact with it. As a result the question arises: What is responsible for this inhibitory effect? There is evidence that the old spleen may be a source of suppression factors [5] and that splenectomy in old animals increases the residual life span in mice of certain lines [2].

The effect of some experimental factors on the primary immune response was studied in animals of different ages connected together in parabiosis.

EXPERIMENTAL METHODS

Female CBA mice of different ages obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used. Operations to form parabiotic pairs were carried out on animals aged 2-3 and 22-25 months under intraperitoneal hexobarbital anesthesia by the method of Bunster and Meyer [4]. The parabiotic pairs were separated surgically two months after the first operation. The immune response in the animals of this group was determined 1 and 3 months later. In another experimental group the spleen of the older partners was removed during the operation for forming parabiotic pairs. The older parabionts of the third group were irradiated with x rays in a dose of 1000 R 3-5 days after joining to the younger animals (RUM-17 apparatus, voltage 180 kV, current 15 mA, filters 0.5 mm Cu + 1 mm Al, dose rate 100 R/min). During irradiation the younger animals were screened. The duration of parabiosis was 2 months. The level of the primary immune response was determined on the fourth day after intraperitoneal injection of $5 \cdot 10^8$ sheep's red blood cells, by counting the number of direct [8] and indirect [6] plaque-forming cells (PFC) in the spleen and by measuring the levels of serum hemolysins and hemagglutinins.

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