METHODS

APPLICATION OF THE SPECIFIC PROTEIN-FIXATION TEST IN TOXOPLASMOSIS

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Toxoplasmosis caused by the protozoan Toxoplasma gondii is associated with a number of serious pathological states (spontaneous abortions, stillbirths, birth of children with developmental defects, early infantile mortality, chorioretinitis, meningoencephalitis and its residual phenomena, myocarditis, interstitial pneumonia, etc. [4, 5]).

There is no single opinion among researchers on many problems of the biology of the parasite and the pathogenesis of the disease [4-6]. The problems of the diagnosis and epidemiology of toxoplasmosis are also disputed [6].

The main diagnostic tests in toxoplasmosis, especially in its latent form, are such immunological tests as the complement-fixation reaction, Sabin-Feldman reaction with a dye, and the intradermal allergic test. These reactions, especially the last, prove to be positive in a rather high percent of cases in healthy people. For example, in a number of countries immunological reactions are positive in more than 60% of the cases among people clinically suspected of having toxoplasmosis, whereas the parasite is detected only in sporadic cases.

The marked dissociation between the parasitological and serological diagnostics justifiably causes the skeptical attitude of many researchers and clinicians toward the specificity of immunodiagnostic tests in toxoplasmosis [7].

In our study we attempted to use for investigation of toxoplasmosis a new immunochemical reaction worked out by Prof. V. S. Gostev and cohorts [1, 2],—the reaction of the specific fixation of serum protein by antigens sorbed on paper (PFT).

The principle of PFT is as follows. A specific complex consisting of antigen and antibody has the capacity to adsorb nonspecific proteins. This method makes it possible to detect a mixture of specific and nonspecific proteins fixed by the antigen-antibody complex.

The greater the activity of the antigen and antiserum, the more protein is fixed by this complex. A control with serum not containing antibodies to a given antigen makes it possible to establish the magnitude of the specifically fixed protein. The result of the reaction is expressed in milligrams of protein with respect to nitrogen [3].

METHOD

In the present study we investigated 25 sera of animals infected and not infected with Toxoplasma gondii: 6 rabbits (4 experimental and 2 control) and 19 rats (13 experimental and 6 control). For comparison we also used 17 sera of persons clinically suspected of having toxoplasmosis.

* Such a dissociation pertains also to investigations of animals for which it is possible to check parasitologically the impressions of the internal organs.

PFT of Antitoxoplasmatic Serum by Antigen Sorbed on Chromatographic Paper

Experimental series	Antiserum	Quant. of protein in 20 mg of sorbed antigen (in μg)	Immune serum	Normal serum	Physiological salt solution	Quant. of protein after incubation	Specific increment of protein
			In ml			In μg	
I	Serum obtained from rabbit No. 2	175	0.3	—	—	525	175
		175	—	0.3	—	350	—
		175	—	—	0.3	175	—
II	Serum obtained from rat No. 16	130	0.3	—	—	393	131
		130	—	0.3	—	262	—
		130	—	—	0.3	130	—
III	Human serum No. 22	175	0.3	—	—	831	262
		175	—	0.3	—	568	—
		175	—	—	0.3	175	—

All cases of animal infection were checked parasitologically and the impressions of the internal organs were studied.

The sorbed test antigens were prepared in the following manner. Samples of chromatographic paper (Whatmann-1) weighing 20 mg each were immersed in a solution of toxoplasmin for 10-15 min, for which the contents of one ampule—25 doses of antigen—was dissolved in 2 ml of physiological salt solution. Fixation of the toxoplasmin was achieved by drying the chromatographic paper impregnated with the antigen at 18-20° for 24 h. Then the sorbed toxoplasmin was washed free from the proteins which were loosely sorbed on the paper. For this purpose the antigen was placed for 45 min in a physiological salt solution which was changed 3 times. The washed pieces of sorbed toxoplasmin were again dried for 24 h at 18-20°, i.e., at room temperature.

The antitoxoplasmatic serum was obtained under sterile conditions by taking blood from the heart of rabbits and rats infected with-Toxoplasma-gondii. The animals were infected by a 4-5 fold subcutaneous injection of an attenuated live culture. Of the 4 rabbits 2 received the live culture in a quantity of 10^6 cells and 2 in a quantity of $20 \cdot 10^6$ cells; 13 rats were infected with a live culture (peritoneal exudate) in a dose ranging from 10^6 to $8 \cdot 10^6$ cells. In the experiment we used sera, the serological analysis of which, carried out by the complement-fixation reaction, yielded a positive result.

The specific protein-fixation test with paper-sorbed toxoplasmin was set up as follows. The sorbed toxoplasmin was placed in a Maksimov chamber (a hollow-ground slide) and covered with 0.3 ml whole antiserum so that the entire antigen was submerged in the serum. At the same time we set up the control test: toxoplasmin with the serum of a healthy animal and toxoplasmin with a physiological salt solution. All tests (3 each) were placed in a moist chamber. The interaction of the sorbed toxoplasmin with the antiserum occurred at room temperature for 1 h in the moist chamber. At the end of incubation the toxoplasmin + antiserum complex was washed free from the loosely fixed proteins, which were mainly serum proteins. For this purpose each test was placed successively in 3 portions of cold physiological salt solution of 10 ml each for 4 min in each. Finally the washed sorbed antigens were transferred to Kjeldahl flasks for combustion with concentration H_2SO_4, after which the nitrogen content was determined in them by Conway's method. The value of the specific fixation of antiserum protein by the antigen sorbed on paper was calculated in micrograms of nitrogen by its difference in the systems: antigen + immune serum and antigen + normal serum.

RESULTS

The sera of animals infected with-Toxoplasma-gondii, as well as a part of the sera of people suspected of toxoplasmosis, manifested a high serological activity which was expressed in an appreciable specific increment of protein. The typical protocols of 3 experimental series are shown in the table.

Serum No. 2 obtained from a rabbit infected with-Toxoplasma-gondii-when interacting with the homologous sorbed test antigen yielded a specific increment of protein equal to 175 μg. Similar results were obtained with other rabbit sera.

The specific increment of protein in the sorbed toxoplasmin in the experiment with sera obtained from rats was 131 μg. Of the 13 infected rats the sera of only 2 did not yield a specific increment; the other 11 sera specifically reacted with the toxoplasmin. The sera of the control animals did not yield a specific increment.

Of the 17 investigated sera of persons clinically suspected of having toxoplasmosis a positive result was obtained in 50% of the cases. The table shows as a example one of the experiments with a positive result. The sorbed toxoplasmin test antigen when reacting with the serum of a patient with a prelinary diagnosis of toxoplasmosis yielded a specific increment, equal to 263 μg.

The investigation of the animals infected with Toxoplasma gondii showed that their sera specifically reacted in almost 90% of the cases in the protein-fixation test with the paper-sorbed toxoplasmin. The sera of persons suspected of having toxoplasmosis yielded to positive results in 50% of the cases. On the basis of the experimental data obtained we consider it possible to recommend the described test for studying the immunology of toxoplasmosis.

SUMMARY

By means of fixation of the antiserum protein with the paper-sorbed antigen (PF) a study was made of the sera of rabbits and rats infected with Toxoplasma gondii as well as of the sera obtained from persons with a preliminary diagnosis of "toxoplasmosis." The results were positive in about 90% of the experiments on the infected animals and in 50% of the persons with the clinically suspected toxoplasmosis.

PFT is recommended for immunological studies of toxoplasmosis.

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