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PRODUCTION OF ANTIBODIES TO INFLUENZA A VIRUS BY HUMAN LYMPHOID CELLS IN VITRO

A. V. Ispolatova, N. L. Korchanova, B. V. Medvenskii, M. S. Pluzhnikov, Al Tamimi Fauzi Akhmed, and Ya. S. Shvartsman

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Reports have recently been published on the possibility of reproducing the production of antibodies against different antigens by human lymphoid cells in culture in vitro. These antigens include xenogeneic erythrocytes and poliomyelitis and influenza viruses. Peripheral blood cells have been used to study antibody production against influenza virus [9, 10, 12].

The method of studying antibody production against influenza virus in vitro can be used to obtain data on the mechanisms of antibody formation and investigations of immunologic memory. A more profound study of the latter problem is necessary with the obtaining of data showing that strain-specific immunity to influenza lasts for a very long time [3, 5]. Memory cells remain in the peripheral blood for several weeks [8]. With these facts and the characteristics of the pathogenesis of influenza in mind, it was decided to study antibody production against influenza A (H3N2) virus by cells of the tonsils and mediastinal lymph nodes – organs draining the region of the "portals of entry" of influenzal infection, where memory cells persist for a long time [2].

EXPERIMENTAL METHOD

Influenza virus A/Leningrad/385/80 (H3N2), grown in a culture of MDCK cells (transplantable canine kidney cells) was used in the experiments. The tonsils and lymph nodes were obtained from adults of both sexes undergoing tonsillectomy for chronic tonsillitis and respiratory diseases. After removal the lymph nodes and tonsils were placed in flasks with Eagle's medium or medium 199 containing 10% bovine serum, 100 units/ml penicillin and streptomycin, and 200 μg/ml kanamycin, and received in the laboratory not later than after 1 h. The same medium was used to wash the cells. Pieces of tissue with no visible signs of inflammation were excised from the tonsils with scissors, transferred to sterile petri dishes with medium, washed 3 times, and again transferred to sterile dishes. Lymph nodes were freed from extraneous tissue and treated in the same way as the tonsils. Cells were isolated from the lymph nodes and tonsils by means of dissection needles, collected in test tubes, and washed twice on the TsLR-1 centrifuge at 800 rpm for 5 min at 4°C. The supernatant after the last washing was collected for determination of antibodies. The residue was resuspended in culture medium.

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TABLE 1. Antibody Production against Influenza Virus by Human Lymphoid Cells in Vitro. Geometric Mean Antibody Titers

Stimulator of antibody production	Tonsil cells					Lymph node cells				
	number	day of culture			P	number	day of culture			P
		0	3rd	7th			0	3rd	7th	
Control		1,07	1,07	1,62	>0,05		1,07	1,81	2,6	>0,05
Influenza A (H3N2) virus	12	—	6,1	4,6	>0,05	7	—	2,5	9,9	<0,05
Typhoid LPS		—	4,0	4,0	>0,05		—	—	—	
P*			<0,05	<0,05					<0,05	

Legend. Asterisk indicates that P was determined by the sign test [1].

For cell culture a mixture was prepared, consisting of Eagle's medium with the addition of deficient components according to the formula for medium RPMI-1640, 10% embryonic calf serum (from the Belorussian Research Institute), and antibiotics. The number of viable cells was counted after staining with trypan blue. The cells were cultured in a chamber consisting of a glass tube 60 mm long and 10 mm in diameter, the bottom end of which was covered by a dialysis membrane, and a test tube 100 mm long and 300 mm in diameter [11]. The tube was fixed inside the test tube so that its lower end was 20 mm above the bottom of the test tube. Medium (9 ml) was poured into the test tube and a suspension of 2×10^6 cells in a volume of 1 ml of medium was introduced into the glass tube. Some suspensions were treated with 64 HA of influenza virus in a volume of 0.2 ml or 25 μg of typhoid lipopolysaccharide (LPS) (Leningrad Research Institute of Vaccines and Sera). Control cultures were treated with 0.2 ml of medium. The chambers were incubated at 37°C in an atmosphere with 5% CO_2. The medium in the test tube was changed every 1-2 days. The indirect hemagglutination test was used to determine antibodies against influenza virus [6]. The test was carried out in modified panels of a Takachi microtitrator, using formalin-stabilized human red blood cells of group O (Rh^-), on the surface of which a suspension of purified HA of influenza virus A (H3N2) was chemically bonded, as the diagnostic serum.

The essence of the modification is that transparent plastic plates 35 mm thick, with holes corresponding to the wells in the panels, were glued to the surface of the panel.

EXPERIMENTAL RESULTS

When modified panels were used the volume of test material was increased to 0.5 ml. In standard panels it is 0.025 ml. In both types of panels the volume of diagnostic serum applied was 0.025 ml. By increasing the volume of test material in systems of antiserum against influenza virus A (H3N2), RS-virus, and type 3 parainfluenza virus with the corresponding erythrocytic diagnostic serum the sensitivity of the reaction in the modified panel was increased by 64-128 times.

Meanwhile the specificity of the test was unchanged. In a system of monospecific serum against influenza B and diagnostic serum prepared from virus A (H3N2), no antibodies could be detected in either standard or modified panels.

The initial viability of the tonsil cells was 85-90% and of the lymph node cells 95%. By the 3rd day of culture 50% of tonsil cells and 80% of lymph node cells remained viable, and on the 7th day the levels were 20 and 70% respectively ($P < 0.01$).

Antibodies against influenza A (H3N2) virus were virtually absent in supernatants obtained after washing the cells and also after their culture in the absence of stimulators (Table 1). Antibody production was observed on stimulation both by influenza virus and by the polyclonal activator LPS. Influenza virus stimulated antibody production more actively than LPS. Differences in the intensity of antibody synthesis to stimulation of tonsil cells by influenza virus and LPS compared with cultures not treated with these antigens are significant ($P < 0.05$).

Antibody synthesis in the presence of LPS can be explained by the ability of the substance to induce polyclonal activation of B lymphocytes, including B memory cells, and also by the property of LPS to replace the function of T lymphocytes during antibody production against influenza virus [4]. The more active antibody production by tonsil cells on the 3rd day than by lymph node cells can most probably be explained by continuous antigenic stimulation of the former. The absence of any increase in antibody concentration on the 7th day of culture of tonsil cells can evidently be attributed to their death.

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