PRODUCTION OF INFLUENZA ANTIBODIES BY LYMPHOCYTES OF THE RESPIRATORY TRACT

E. N. Agranovskaya, A. I. Mal'tseva, Ya. S. Shvartsman, and L. V. Solilova-Bozhenko

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Antibody production against influenza virus by lymphocytes of the human nasopharyngeal tonsils and the tracheal wall of rats was investigated. Antibody-producing cells were found in preparations of lymphadenoid tissue removed in the postepidemic period. Intranasal immunization of rats with living influenza vaccine led to the accumulation of producer cells in the tracheal wall and of antibodies in the secretions of the respiratory tract. Reimmunization was followed by a well-marked secondary response. A characteristic feature of secretory immunity is the slower formation of producer cells and of antibodies than is observed in the system of general immunity.

KEY WORDS: antibodies; lymphocytes; influenza; respiratory tract.

The concept of two systems of specific humoral immunity has been formed in recent years. The first system is responsible for the production of antibodies contained in the blood serum and other tissues of the internal milieu, the second for the synthesis of antibodies present in the external secretions and excretions [4-7].

Most of the secretory antibodies are synthesized by lymphocytes located in the walls of organs communicating directly with the external environment [3, 4]. Meanwhile, the development of the plasma-cell response and the dynamics of accumulation of the antibody-producing cells in the mucosa and submucosa of the respiratory and alimentary tracts have been inadequately studied.

The production of influenza antibodies by the cells of the lymphadenoid tissue of unimmunized children and the trachea of animals immunized with influenza virus was investigated.

EXPERIMENTAL METHOD

Lymphadenoid tissue was obtained from children undergoing operations for hypertrophy of the nasopharyngeal tonsils; 140 Wistar rats weighing 120-150 g were immunized intranasally under superficial ether anesthesia with 0.8 ml of an allantoic culture of A/Hong Kong/1/68/21 (H3N2) influenza virus diluted 1:10 with 6% peptone solution. Instead of the virus, the rats of the control group received peptone solution. The animals were sacrificed in groups of 8-10 before and at various times after immunization. Blood and mucus from the respiratory organs were collected, and the trachea, the lymph gland and its bifurcation, and the spleen were removed. The trachea was divided longitudinally and the mucosa and submucosa were removed with a scalpel and transfered into medium No. 199. To obtain cell suspensions the tissue was teased apart with dissecting needles and then filtered through Kapron gauze. Human (for testing lymphadenoid tissue cells) or sheep's (for testing the animal cells) red cells, conjugated with influenza virus by chemical bonds [1], were used as the test antigen for determining the number of producer cells. The same preparation was used to determine antibodies in the indirect hemagglutination test (IHT). To detect producer cells

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TABLE 1. Determination of Cells Producing Antibodies against A/Hong Kong/1/68 Influenza Virus in Human Lymphadenoid Tissue

Time of operation	No. of cells investigated	No. of active cells	
		abs.	%
Before influenza epidemic	1280	4	0,3
	1556	2	0,1
	900	4	0,4
	1300	4	0,3
After influenza epidemic	1194	5	0,4
	1550	14	0,9
	1820	13	0,7
	1264	10	0,8

a drop of suspension containing 10^5 cells/ml was added to a drop of a broth culture of a nonpathogenic streptococcus and an equal volume of a suspension of red cells either sensitized or not sensitized with the virus. The mixture was kept for 30 min at room temperature and interaction of the cells with the test antigen studied with the aid of a phase-contrast optical system [2]. Cells with the morphological characteristics of plasma cells but without phagocytic activity and fixing not less than 3 red cells on their surface were taken as producers. When the results of the experiments were read the number of cells interacting with unsensitized erythrocytes was subtracted from the number of cells fixing sensitized red cells. The difference thus obtained gave the number of cells producing antibodies against influenza virus in the material studied.

To study the plasma-cell response in the trachea and lymphoid tissue the organs were fixed in Carnoy's fluid. Sections were stained by Brachet's method. The intensity of development of the plasma-cell responses was estimated in sections through the lymphoid organs and trachea at the sites of the greatest concentrations of plasma cells in 50 fields of vision.

The results were subjected to statistical analysis, using Wilcoxon's criterion, by the Minsk-22 computer in the Laboratory of General Epidemiology of the Institute.

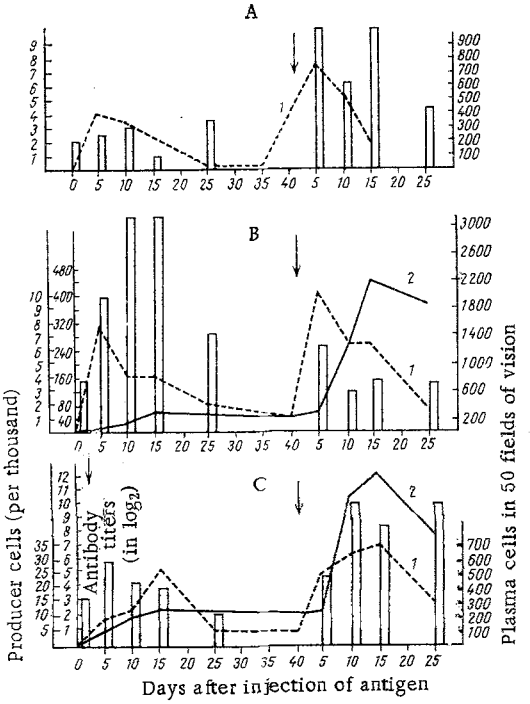

Fig. 1. Dynamics of antibody accumulation in secretion of respiratory tract and blood serum, and of producer cells and plasma cells in the tracheal wall and lymphoid organs of rats immunized with A/Hong Kong/1/68 influenza virus: A) producer cells and plasma cells in the spleen; B) producer cells and plasma cells in the lymph gland at the tracheal bifurcation and antibodies in the blood serum; C) producer cells and plasma cells in the tracheal wall, antibodies in the secretion of the respiratory tract. 1) Antibody-producing cells; 2) antibody titers. Columns denote plasma cells. Arrow marks injection of antigen.

EXPERIMENTAL RESULTS

Investigation of 8 preparations of lymphadenoid tissue revealed a significant ($P < 0.05$) increase in the number of active cells in the material obtained after the end of an influenza epidemic (Table 1).

A marked increase in the antibody concentration in the blood serum of the immunized rats was observed after the 5th day, coinciding with the maximal increase in the number of active cells in the lymph gland draining the trachea and in the spleen (Fig. 1).

By contrast, a significant increase in the antibody concentrations in the secretion after a single immunization was observed for the first time only on the 10th day; the number of plasma cells in the tracheal wall was unchanged and the rate of increase in the number of producer cells was slower. This index reached its maximum by the 15th day. Repeated antigen stimulation led to a much more active accumulation of plasma cells and producer cells in the tracheal mucosa and submucosa and of antibodies in the secretion from the mucous glands of the trachea. Meanwhile the number of producer cells in the tissue of the lymph gland at the bifurcation of the trachea and of the spleen increased and there was a sharp rise in the antibody concentration in the blood serum.

The results thus confirmed observations concerning the local synthesis of all or most of the secretory antibodies. Evidence in support of this conclusion is given by the good agreement between the dynamics of their accumulation in the lumen of the trachea and the formation of plasma cells and producer cells in the wall of that organ. The presence of a secondary response in the system for the synthesis of secretory antibodies indicates the value of repeated injections of viral and bacterial vaccines in intranasal, aerosol, and enteral immunization. In order to establish the optimal intervals for this revaccination, the pattern of development of the immunologic memory in the system of secretory immunity must be investigated. A more intensive study is also required of the causes of the much greater relative number of antibody-producing cells in the trachea than in the lymph gland and spleen. This is evidently explained by migration of precursor cells capable of interacting with the immunogen into the focus of antigenic stimulation.

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