in the lung tissue. Whereas after intraperitoneal infection suppression of the process is probably due to an increase in antiendotoxic resistance [7-8], in a developed infectious process following a progressive course (lung model) this stimulation was evidently less effective.

When induction of nonspecific resistance is studied, separate attention must therefore be paid to the conditions responsible for antitoxic and antiinfectious resistance. The ability of endotoxins to stimulate nonspecific resistance is determined by their high-molecular-weight component.

LITERATURE CITED

1. I. P. Ashmarin and A. A. Vorob'ev, Statistical Methods in Microbiological Research [in Russian], Leningrad (1962).
2. O. V. Bukharin, "Stimulation of natural resistance of the organism to infection by drugs," Author's Abstract of Doctoral Dissertation, Leningrad (1969).
3. T. G. Vasil'eva, Dokl. Akad. Nauk SSSR, 184, 225 (1969).
4. T. G. Vasil'eva, G. I. Sazonets, and N. I. Shapiro, Dokl. Akad. Nauk SSSR, 221, 711 (1973).
5. M. V. Voino-Yasenetskii and M. K. Voino-Yasenetskaya, in: Problems in Infectious Pathology [in Russian], Leningrad (1962), p. 19.
6. M. V. Voino-Yasenetskii and T. N. Khavkin, Zh. Mikrobiol., No. 4, 98 (1964).
7. Z. V. Ermol'eva and G. E. Vaisberg, Stimulation of Nonspecific Resistance of the Organism and Bacterial Polysaccharides [in Russian], Moscow (1976).
8. A. S. Tolybekov, in: The Use of Laboratory Animals in the Development, Production, and Control of Biological Medical Preparations (Abstracts of Proceedings of a Conference) [in Russian], Moscow (1976), p. 117.
9. N. I. Shapiro, T. G. Vasil'eva, I. V. Moskvicheva, et al., Zh. Mikrobiol., No. 11, 35 (1971).
10. N. I. Shapiro, T. G. Vasil'eva, and T. M. Kolotinskaya, Zh. Mikrobiol., No. 4, 120 (1976).
11. S. L. Yagud, "The study of the immunogenic properties of *Shigella sonnei*," Author's Abstract of Candidate's Dissertation, Kiev (1959).
12. W. Braun and M. Nakano, Proc. Soc. Exp. Biol. (New York), 119, 701 (1965).
13. M. Landy and L. Pillemer, J. Exp. Med., 104, 383 (1956).
14. H. Raistrick and W. Topley, Br. J. Exp. Pathol., 15, 113 (1934).

EFFECT OF INFLUENZA VIRUS AND ITS STRUCTURAL COMPONENTS ON THE IMMUNOCOMPETENT SYSTEM OF ANIMALS

N. L. Novichenko, V. I. Struk, and R. E. Kavetskii*

UDC 616.988.75-07:612.017.11

The effect of influenza A ($PR_8/34$) virus and its structural components on immunologic reactivity was studied in mice. Neuraminidase, the enzyme of the influenza virus outer membrane, possesses an immunodepressive action. The addition of neuraminidase leads to removal of sialic acids from the surface of the lymphocytes and reduces their electrophoretic mobility. The mechanism of the immunodepressive action of neuraminidase is discussed.

KEY WORDS: *influenza virus; immunodepression; neuraminidase; lymphocytes.*

The pathogenesis of influenza infection and the complications accompanying it have not yet been properly explained. In recent years immunologic changes in influenza have been

*Academician of the Academy of Sciences of the Ukrainian SSR.

Laboratory of Virus Carcinogenesis, Department of General Mechanisms of Carcinogenesis, Institute for Problems in Oncology, Academy of Sciences of the Ukrainian SSR, Kiev.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 5, pp. 547-549, May, 1978. Original article submitted October 24, 1977.

described. In particular, in experimental influenza in albino mice marked inhibition of antibody formation in response to injection of the test antigen has been demonstrated [2].

The object of this investigation was to study the effect of the various structural components of influenza virus on the immunocompetent system.

EXPERIMENTAL METHODS

Influenza virus A ($PR_8/34$) (H_0/N_1), adapted to mice, was used. The virus was cultured in 9- to 11-day chick embryos for 48 h at 37°C. Tests were carried out on BALB/c mice aged 2 months. In each experiment 10 to 15 mice were used. The preparations for testing were injected 24 h before the test antigen. Immunologic reactivity and the degree of immunodepression were determined on the 5th day after injection of the test antigen by the method of Jerne and Nordin [6] in the modification [1].

Influenza virus was purified by a combination of differential isodensity and rate-zonal centrifugation. The S and V antigens were separated by disintegration of the virus with ether. S Antigen was obtained by removal of the V antigen by adsorption on chick erythrocytes; V antigen was obtained by sedimentation of the S antigen by ultracentrifugation (100,000g) for 2 h. Neuraminidase was isolated from V antigen by treatment with 1% sodium dodecyl sulfate [7]. The content of sialic acids was determined by a thiobarbiturate method [9]. The cytotoxic test and determination of the electrophoretic mobility of the lymphocytes were carried out by methods described in [3, 5].

EXPERIMENTAL RESULTS

In the experiments of series I the immunodepressive action of whole influenza A ($PR_8/34$) virus, after disintegration by ether into S and V antigens, and also the action of S antigen, V antigen, and neuraminidase separately were compared. The original virus in a titer $10^{6.5}$ EID_{50} was injected in a dose of 0.2 ml into the mice. The structural components were injected in doses equivalent as protein to their content in 0.2 ml whole virus in a dose of $10^{6.5}$ EID_{50}. Although the immunodepressive action of influenza virus on mice is well marked by intranasal injection of 10^3 EID_{50} [2], the high titer of virus and the intraperitoneal method of injection were chosen in order to elucidate precisely the role of the structural components of influenza virus in immunodepression.

Data on the effect of infectious virus and its structural components on the number of antibody-forming cells (AFC) were subjected to statistical analysis and the results are given in Fig. 1.

The results indicate that the neuraminidase, the enzyme of the outer membrane of the influenza virus, possesses an immuodepressive action. The remaining components had no effect on immunologic reactivity. Neuraminidase, inactivated by heating, likewise had no immunodepressive action. These results are in agreement with those showing the immunodepressive action of neuraminidase of influenza virus on rat lymphocytes [11].

Considering the abundant evidence of the increased immunogenicity of cells after treatment with neuraminidase from *Vibrio cholerae* [10], the surface properties of mouse spleen lymphocytes were investigated 5 days after intraperitoneal injection of 0.02 μg neuraminidase

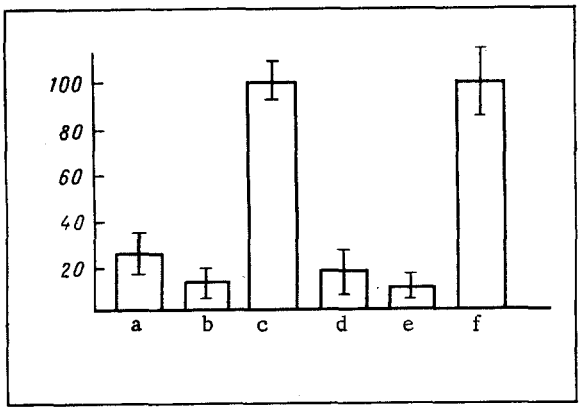

Fig. 1. Effect of influenza A ($PR_8/34$) (H_0/N_1) virus and its structural components on the number of AFC. Abscissa: a) whole virus; b) virus split into S and V antigens; c) S antigen; d) V antigen; e) neuraminidase; f) neuraminidase inactivated by heating; ordinate, number of AFC per 10^6 nucleated spleen cells (in % of control).

TABLE 1. Viability of Lymphocytes from Intact Mice and Mice Treated with Neuraminidase

Antigen	Antiserum	Complement	Lysis, %	P
NAL	NAS	+	81,6	<0,01
NAL	NAS	—	0	
NAL	IS	+	80,7	<0,01
NAL	IS	—	0	
IL	NAS	+	0	
IL	NAS	—	0	
IL	IS	+	0	

Note. NAL) Lymphocytes of mice treated with neuraminidase; NAS) serum of mice treated with neuraminidase; IL) lymphocytes of intact mice; IS) serum of intact mice.

(the peak of immunodepression). Lymphocytes from experimental and control animals were treated with preparations of influenza virus neuraminidase containing 1.7 units of enzyme activity; under these circumstances 0.06 and 0.28 μg sialic acids per 10^6 cells was removed. The results of the study of electrophoretic mobility of the lymphocytes are given in Fig. 2.

The results point to a marked decrease in the surface charge of the lymphocytes of the mice receiving neuraminidase.

Removal of sialic acids from the surface of the cells exposes latent antigenic determinants, and a decrease in the surface charge on regions rich in receptors containing sialic acids affects the configuration of neighboring antigenic determinants not containing sialic acids [10].

As part of the study of the action of neuraminidase on lymphocytes the cytotoxic test was carried out with different combinations of antigens and antisera. The results of the cytotoxic test are given in Table 1.

The results show that normal mouse serum contains natural antibodies against lymphocytes treated in vivo with neuraminidase. The sera of such mice have no cytotoxic properties against normal lymphocytes. Similar results were obtained by Rogentine and Plocinic [8], who studied the presence of natural antibodies against autologous lymphocytes, treated with neuraminidase from *V. Cholerae in vitro*, in human serum.

The results now obtained, together with data in the literature [4] thus explain the immunodepressive action of influenza virus in this particular model by the acquisition of autoantigenic properties by the lymphocytes and by the cytotoxic action of normal serum.

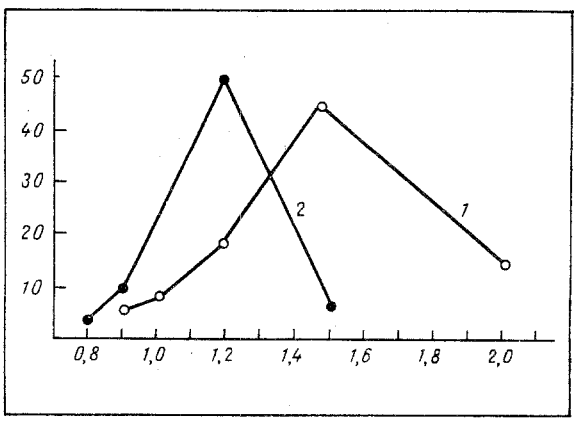

Fig. 2. Electrophoretic mobility of normal mouse lymphocytes (1) and lymphocytes of mice treated with neuraminidase (2). Neuraminidase (0.02 μg) was injected intraperitoneally 5 days before the experiment. Abscissa) electrophoretic mobility (in $cm^2 \cdot V^{-1} \cdot sec^{-1} \cdot 10^{-4}$); ordinate) number of lymphocytes.

LITERATURE CITED

1. L. A. Pevnitskii, V. V. Solov'ev, and L. N. Fontalin, Byull. Éksp. Biol. Med., No. 8, 85 (1965).
2. L. V. Yakimenko, Z. D. Savtsova, S. L. Rybalko, et al., Vopr. Virusol., No. 1, 96, (1975).
3. H. A. Abramson, L. S. Moyer, and M. H. Gorin, Electrophoresis of Proteins, New York (1942), pp. 126-128.
4. R. C. Hughes, P. D. Palmer, and B. H. Sandford, J. Immunol., 111, 1071 (1973).
5. P. Isaacson, Prog. Allergy, 10, 256 (1976).
6. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
7. W. G. Laver and K. C. Valentine, Virology, 38, 105 (1969).
8. G. N. Rogentine and B. A. Plocinic, J. Immunol., 113, 848 (1974).
9. G. Warren, J. Biol. Chem., 237, 1971 (1959).
10. G. Weiss, J. Natl. Cancer Inst., 50, 3 (1973).
11. J. J. Woodruff and J. F. Woodruff, J. Immunol., 117, 859 (1975).

STUDY OF THE CHEMICAL RECEPTOR FOR DIPHTHERIA TOXIN IN CELL MEMBRANES

Yu. V. Vertiev and Yu. V. Ezepchuk

UDC 576.852.23.097.29.095.18:547.952

Adsorption of diphtheria toxin on immobilized preparations of cell membranes was studied in the presence of gangliosides. A mixture of gangliosides completely suppresses adsorption of the toxin on membranes of cells both sensitive (HeLa cells, macrophages) and resistant (L cells) to its action. Gangliosides treated with neuraminidase are less effective. Immobilized gangliosides effectively adsorb diphtheria toxin in the presence of protective colloid and of the detergent Triton X-100. On the basis of these results gangliosides can be regarded as the receptors of diphtheria toxin.

KEY WORDS: *diphtheria toxin; receptors; membranes; gangliosides.*

The chemical nature of cell receptors has recently been established for a number of bacterial toxins. For cholera, staphylococcal, tetanus, and botulinus toxins gangliosides have been shown to perform the receptor function in the cell [6, 8-10, 11, 13]. Data on the receptor for diphtheria toxin are not available, although the molecular structure and mechanism of action of this toxin have been studied in much greater detail than those of other toxins [3]. Meanwhile, experiments using the "viroimmunotest" enabled the writers to show that diphtheria toxin is fixed on cell membranes [2].

The object of this investigation was to study the effect of gangliosides on interaction between diphtheria toxin and cell membranes.

EXPERIMENTAL METHODS

Diphtheria toxin was obtained by the method of Gill and Dinius [7] and the concentration of its toxic protein was determined spectrophotometrically [12]. Membranes from HeLa and L cells and from guinea pig macrophages were obtained and immobilized on Sephadex G-25 by the method described previously [2]. Bovine serum albumin (from Koch-Light, England) and gangliosides (from Sigma, USA) were immobilized on Sephadex G-25 by Cuatrecasas' method [5] and designated Seph–BSA (Sephadex–bovine serum albumin) and Seph–BSA–G (Sephadex–bovine serum albumin–gangliosides), respectively. A purified preparation of neuraminidase from NAG vibrio [1] was immobilized on Sepharose 4B (from Pharmacia, Sweden) by Cuatrecasas' method [4]. The

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 5, pp. 549-551, May, 1978. Original article submitted April 7, 1977.

 0007-4888/78/8505-0598$07.50