REACTIONS OF LYMPHOCYTES AND MACROPHAGES OF GUINEA PIGS TO TOXIC ACTION OF INFLUENZA VIRUS

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UDC 612.42.014.46:576.858.75.097.29

Influenza viruses differing in their virulence to man have different effects on lymphocytes and macrophages in the peritoneal exudate of intraperitoneally infected guinea pigs. With strengthening of the virulent properties of influenza viruses for man, their inhibitory action on guinea pig lymphocytes and macrophages also intensifies. Weakly virulent strains of influenza viruses cause a substantial increase in the functional activity of macrophages but they have no lympholytic properties. Since differences in the cell composition of the exudate and in the functional state of the macrophages correlated with the degree of virulence of the viruses to man, the study of responses of lymphocytes and macrophages of animals resistant to influenza virus can be used to determine the toxic properties of test viruses.

KEY WORDS: Influenza viruses; virulence; reactions of lymphocytes and macrophages.

The results of an assessment of the toxic action of vesicular stomatitis virus on the state of lymphocytes and macrophages in the peritoneal exudate of mice have been published previously [8, 9].

In the investigation now described the toxic action of strains of influenza virus of different virulence to man on lymphocytes and macrophages of the peritoneal exudate of guinea pigs infected intraperitoneally was studied. It was assumed that under these conditions of infection influenza virus does not reproduce in guinea pigs; the changes observed could accordingly be largely the result of the toxic action of the virus [11].

EXPERIMENTAL METHOD

Guinea pigs weighing 250 g were used. The following strains of influenza A/Hong Kong/68 virus were used: 1) vaccine for children, 2) virus A/Hong Kong/68 (original) with average virulence, and also viruses A/Victoria/72 and A/Leningrad/72, highly virulent to man. All strains of influenza virus were injected intraperitoneally 1 and 24 h before obtaining the exudate from the peritoneal cavity in a dose of 8.0-9.0 log EID_{50} and a volume of 3 ml. Control animals were injected with allantoic fluid from uninfected chick embryos under the same conditions.

At autopsy on the animals killed with ether, peritoneal exudate was obtained by the method of Il'in et al. [4]. The cells were counted in a Goryaev's chamber. The general morphology of the cells was studied in films stained by the Giemsa method and their functional state was estimated from the acid phosphatase activity, determined by Burtyanskii's method [1] after staining by Burstone's method [10]. To detect influenza virus in macrophages and lymphocytes the films were fixed in acetone, stained by the direct Coons' method and the intensity of fluorescence was determined under the ML-4 microscope, from 20 to 50 cells in each film being counted.

Laboratory of Pathogenesis and Pathomorphology and Laboratory of the Etiology of Influenza and Acute Respiratory Diseases, All-Union Research Institute of Influenza, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. A. Smorodintsev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 4, pp. 497-499, April, 1976. Original article submitted February 28, 1875.

TABLE 1. Composition of Peritoneal Exudate and Functional Activity of Macrophages 24 h after Intraperitoneal Injection of A Influenza Viruses Differing in Their Virulence to Man (M ± m)

Group No.	Material injected intraperitoneally	No. of mononuclear cells (in millions/ml)				Histochemical indices of acid phosphatase content in macrophages			
		lymphocytes		macrophages		HIC*		mean number of granules per cell	
1	Control (before injection)	3,6±1,0	—	0,15±0,1	—	0,3±0,09	—	1,8±0,8	—
2	Placebo P_{1-2}	6,3±1,5	<0,01	0,9±0,4	<0,01	0,47±0,07	<0,01	4,1±0,8	<0,01
3	A/Hong Kong/68 virus (original) P_{2-3}	3,0±1,3	<0,05	5,0±1,8	<0,01	0,59±0,09	>0,05	5,1±1,0	>0,05
4	A/Victoria/72 virus P_{3-4}	0,7±0,5	<0,01	4,8±1,2	>0,05	0,4±0,11	<0,01	3,5±1,7	>0,05
5	A/Leningrad/72 virus P_{3-5}	1,4±1,0	<0,01	3,6±1,9	>0,05	0,19±0,05	<0,01	1,4±0,5	<0,05
6	A/Hong Kong/68 virus (vaccine for children) P_{3-6}	6,6±1,9	<0,01	5,6±2,2	>0,05	1,17±0,14	<0,01	14,0±2,0	<0,01

*Histochemical index of content.

The numerical results were subjected to statistical analysis by Wilcoxon's method [2], in consultation with Yu. G. Ivannikov, Head of the Laboratory of General Epidemiology.

EXPERIMENTAL RESULTS

In the study of lymphocytes and macrophages of infected guinea pigs by the immunofluorescent method no influenza virus or its antigens could be found in the lymphocytes. In the macrophages 1 h after intraperitoneal injection of A/Hong Kong/68 and A/Victoria/72 viruses a statistically significant increase in the intensity of specific fluorescence was observed (0.15 ± 0.01 and 0.09 ± 0.02 in the experimental and 0.04 ± 0.01 in the control series); after 24 h the intensity was reduced in the case of infection with A/Hong Kong/68 virus and there was no increase at all in the case of virus A/Victoria/72.

Despite the absence of specific fluorescence in the lymphocyte and in most of the macrophages after 24 h, the exudate obtained after injection of viruses with different levels of virulence to man differed considerably in cell composition and in functional activity of the phagocytes studied (Table 1).

In most cases intraperitoneal injection of influenza viruses into guinea pigs led to a substantial decrease in the number of lymphocytes compared with their number in the control animals receiving the placebo. The lympholytic effect increased with an increase in the virulence of the virus. It was found after injection of the original strain of A/Hong Kong/68 virus and it rose sharply following injection of A/Victoria/72. The vaccine for children was an exception, for it caused changes similar to those produced by the placebo.

Counting the number of macrophages showed that the influenza viruses caused the development of a marked macrophagal response. The number of macrophages in the experimental groups rose significantly compared with the control receiving the placebo. However, this increase in the number of macrophages after injection of viruses with increased virulence was accompanied by inhibition of their functional activity; when viruses of low virulence were used the enzyme activity of the phagocytes was significantly increased.

Considering that differences in the cell composition of the exudate and in the functional state of the macrophages correlated with the level of virulence of the viruses to man, that influenza virus did not reproduce in the guinea pigs and its stay in the phagocytic cells was of short duration, and that no virus was found in the lymphocytes, it is suggested that the changes observed were due to the toxic properties of the viruses.

According to the literature influenza virus can inhibit phagocytosis, and this has been observed even when the virus could not be found in the cells or was present in very small amounts. Accordingly most workers consider that a decrease in the intensity of phagocytosis is one manifestation of the toxic properties of influenza virus [3, 5-7].

Another essential factor for the assessment of the toxic properties of the influenza virus is assessment of macrophage function as reflected in the activity of hydrolytic enzymes, which correlates closely with the indices of phagocytosis [6, 7]. Different toxins, including the toxins of influenza virus, inhibit both

phagocytic and enzymic activity [6, 7, 12]. Moreover, influenza patients frequently develop lymphocytopenia. In the present experiments definite changes were observed in the composition of both the lymphocytes and the macrophages of the exudate, which depended on the degree of virulence of the viruses used.

It can be concluded from these results that the study of the responses of lymphocytes and macrophages in animals resistant to influenza virus could be used to determine the toxic properties of test viruses.

LITERATURE CITED

1. D. A. Burtyanskii, Lab. Delo, No. 3, 70 (1961).
2. F. Wilcoxon, cited by B. L. Van der Waerden, Mathematical Statistics [Russian translation], Moscow (1960), p. 328.
3. L. Ya. Zakstel'skaya, The Toxicity of Influenza Virus [in Russian], Moscow (1953).
4. G. I. Il'in, A. A. Kyazimova, and T. I. Fomina, in: Collected Scientific Transactions of the All-Union Research Institute of Influenza [in Russian], Vol. 1, Leningrad (1969), pp. 93-102.
5. P. N. Kiselev, The Toxicology of Infectious Processes [in Russian], Leningrad (1971).
6. I. V. Skards, Activation of Leukocytes [in Russian], Riga (1968).
7. L. V. Solilova, A. A. Kyazimova, G. I. Il'in, et al., in: Problems in Virology and the Pathogenesis of Respiratory Virus Infections [in Russian], Leningrad (1970), pp. 56-64.
8. L. V. Solilova, G. I. Il'in, and A. A. Kyazimova, in: Collected Transactions of the All-Union Research Institute of Influenza [in Russian], No. 2, Leningrad (1971), pp. 67-73.
9. L. V. Solilova, G. I. Il'in, A. A. Kyazimova, et al., Vopr. Virusol., No. 6, 670 (1970).
10. M. Burstone, cited by Z. G. E. Pearse, Histochemistry, Theoretical and Applied [Russian translation], Moscow (1962), p. 812.
11. C. Voisan, G. Martin, et al., C. R. Acad. Sci. (Paris), 249, 2425 (1959).
12. G. Weismann, H. Keiser, and A. Bernheimer, J. Exp. Med., 18, 205 (1963).