Acta virol. **26**: 474—480, 1982

CHARACTERISTICS OF CELL-MEDIATED IMMUNITY AFTER ORAL ADMINISTRATION OF A LIVE INFLUENZA VACCINE

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Received January 12, 1982; revised May 12, 1982

Summary. — Development of immunological status of children after oral administration of a live influenza vaccine was followed using different tests of the cellular (blast transformation assay, identification of T, B and nil lymphocytes) and humoral (haemagglutination inhibition test, neuraminidase-inhibition test, neutralization test) immune response. Direct correlation was observed between the increase of neutralizing antibody and the lymphocyte stimulation indices in blast transformation assay (BTA). In vaccinated children the reactivity of lymphocytes was reduced and the amount of T lymphocytes decreased. The influenza vaccine has been shown to possess weak sensibilizing properties.

Key words: *influenza virus*; *influenza vaccine*; *immune system*; *cellular immunity*; *T, B and nil lymphocytes*; *allergy*

Introduction

At present, much attention is focused on the study of mechanisms of immunity. It is connected with the necessity to increase the immunogenicity of vaccines and with the problem of sensibilization.

In this respect the knowledge of subtle mechanisms of antiviral immunity, including antiinfluenza immunity, and usage of tests applied for a more comprehensive characterization of the immune system in vaccinated children are of great importance for evaluation of the efficacy of recent influenza vaccines. The present paper described some characteristics of the immune system after oral administration of a live influenza vaccine.

Materials and Methods

Vaccine administration. Fourty-five 8-9 years old children showing no contraindications to vaccination were included into study. All children were divided into 2 groups: group 1 (vaccinated) comprised 30 children which were vaccinated three times in the interval of 10 days; they were inoculated orally with 2 ml of live influenza vaccine strain A/M/1849/80 (H3N2) obtained by successive passages in suspension of avian embryo tissue cultures (infectious titre $10^{7.5}$ EID_{50}/ml). Group 2 (control) consisted of 15 children which three times received placebo. The batch of live influenza vaccine employed was obtained by inoculation of the influenza virus vaccine strain

in Japanese quail cell cultures from which the medium was harvested and lyophilized in the presence of a stabilizer. The vaccine was prepared in Influenza Vaccines Laboratory headed by Prof. A. K. Alekseeva (Moscow Research Institute for Viral Preparations).

Studies of antigenic potency. To study the antigenic potency of the vaccine, sera were taken from children prior to vaccination and on days 10, 20, 30 and 50 following the first vaccination dose, and assayed in the following tests: haemagglutination-inhibition (HI),neutralization in chick embryos (Nt) and neuraminidase-inhibition (NI) using conventional techniques (Gorbunova, Sokolov, 1960; Aminoff, 1961).

Blast transformation assay (BTA). BTA was studied in the dynamics prior to, and on days 10, 20, 30 and 50 following vaccination. For specific BTA the live uninactivated purified influenza A virus (H3N2) (titre of $10^{6.0}$ EID_{50}/ml) was used as specific antigen. Allantoic fluid from uninfected chick embryos obtained in the same periods of incubation, was used as uninfected control, while rabies virus was used as heterologous control antigen. For unspecific BTA, phytohaemagglutinin (PHA) (Institute of Infectious and Parasitary Diseases, Sofia) was used as mitogen at a concentration of 25 μg per sample (the dose was determined in preliminary studies). BTA was performed according to a radiometric microvariant utilizing whole blood cells according to the method of Pauly *et al.* (1973). The stimulation index (SI) was calculated using the formula:

$$SI = \frac{\text{number of disientegrations/min in the presence of mitogen (antigen)}}{\text{number of disintegrations/min in the absence of mitogen (antigen)}}$$

Identification of T, B and nil lymphocytes. T, B and nil lymphocytes were assayed prior to, and on days 10, 20, 30 and 50 after vaccination using the method of Ivanova *et al.* (1979). The ratios of the main immunocompetent subpopulations of T, B and nil lymphocytes were determined by means of acid phosphatase staining according to the method of azocombination using naphthol--As-diphosphate and dinitrogated pararosaniline. For evaluation of the results the indices of lymphocyte subpopulations expressed per cent, were converted to absolute values.

Blood cell counts. Leukocyte count and leukocyte formulas were determined using conventional techniques in all children prior to vaccination and on days 10, 20, 30 and 50 after it. To evaluate the data obtained, indices of the leukocyte formula expressed per cent were converted to absolute values.

Neutrophils impairment index (NII). NII was determined in various times — prior to vaccination and on days 10, 20, 30 and 50 after the first vaccination using the method of Fradkin *et al.* (1962). Statistic evaluation of the results obtained was carried out using the techniques of variation statistics (Urbach, 1963).

Results

Blast transformation assay (BTA)

In the first series of experiments we aimed to elucidate the degree of sensibilization of lymphocytes in the process of immunity formation after antiinfluenza vaccination. BTA was used for this purpose, and a parallel stimulation of lymphocytes with a nonspecific PHA mitogen allowed us to judge about the functional activity of the T-system. With specific antigen (influenza virus), the index of stimulation of lymphocytes in the vaccinated and control groups prior to vaccination was 1.81 ± 0.14 and 1.48 ± 0.28 respectively. In the vaccinated group the index of stimulation was significantly increased after vaccination, reaching the value 11.87 ± 2.83 on day 20. Then the index decreased, but even 50 days after the vaccination it remained as high as 5.50 ± 1.50. No proliferative stimulation of lymphocytes with specific antigen was observed in the control children (Table 1).

Table 1. Specific blast transformation of lymphocytes in vaccinated children

Children		SI (M ± m)				
Group	No. examined	Prior to vaccination	After vaccination (days)			
			10	20	30	50
Vaccinated	30	1.81 ± 0.14	6.82 ± 1.70	1.87 ± 2.83	5.50 ± 1.30	5.50 ± 1.50
Control	15	1.48 ± 0.28	1.86 ± 0.20	1.78 ± 0.21	1.72 ± 0.20	1.64 ± 0.35

Table 2. Non-specific blast transformation of lymphocytes in vaccinated children

Children		SI (M ± m)				
Group	No. examined	Prior to vaccination	After vaccination (days)			
			10	20	30	50
Vaccinated	30	437.08 ± 77.88	255.44 ± 55.14	112.56 ± 16.82	382.61 ± 71.93	428.45 ± 97.32
Control	15	467.15 ± 98.32	418.71 ± 118.81	482.47 ± 69.61	430.58 ± 91.61	463.37 ± 139.22

Table 3. Absolute counts of T, B and nil lymphocytes in peripheral blood of vaccinated children

Children Group	No. examined	Lymphocytes	Prior to vaccination	After vaccination (days)			
				10	20	30	50
Vaccinated	30	T	1556.3 ± 50.1*	1220.6 ± 66.7	1218.3 ± 46.8	924.9 ± 53.9	1415.1 ± 50.1
Control	15		1235.3 ± 42.3	1193.8 ± 79.3	1248.5 ± 77.7	1202.2 ± 64.0	1279.8 ± 87.7
Vaccinated	30	B	431.3 ± 13.9	366.4 ± 20.0	291.4 ± 11.2	310.3 ± 18.1	416.6 ± 14.8
Control	15		396.2 ± 28.3	355.1 ± 23.6	359.1 ± 22.3	357.7 ± 19.0	382.7 ± 26.2
Vaccinated	30	Nil	1628.9 ± 52.4	1255.8 ± 68.6	1344.1 ± 51.7	1354.4 ± 78.9	1457.5 ± 51.6
Control	15		1312.6 ± 49.0	1252.2 ± 83.2	1352.1 ± 84.1	1263.4 ± 67.3	1365.2 ± 93.5

* per μl

Table 4. Serum antibodies in vaccinated children

Test	No. of children examined	Prior to vaccination	After vaccination (days)				Seroconversion, %
			10	20	30	50	
HI	30	4.79 ± 0.19*	5.89 ± 0.14	6.11 ± 0.08	6.23 ± 0.11	6.90 ± 0.16	76.67
NI	30	2.87 ± 0.09	4.61 ± 0.15	5.53 ± 0.10	5.65 ± 0.12	5.72 ± 0.10	83.33
Nt	30	1.75 ± 0.08	2.45 ± 0.17	3.63 ± 0.15	4.57 ± 0.18	7.71 ± 0.15	73.33

* $\log_1$ of the geometric mean titre (M ± m)

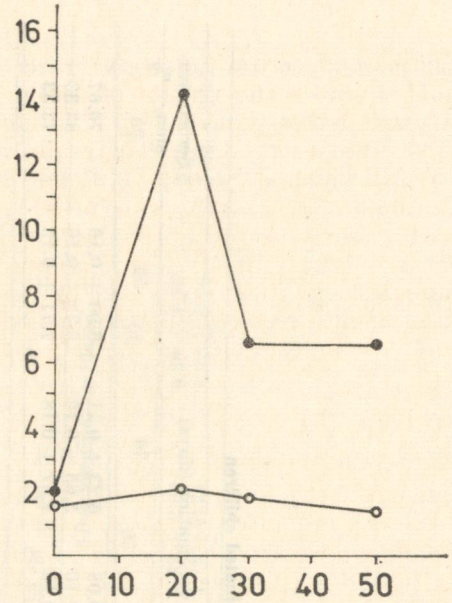

Fig. 1.

Dynamics of specific BTA in vaccinated children as related to neutralizing antibodies
1 — Children with a 4-fold and higher rise of virus neutralizing antibody; 2 — children with no rise of virus neutralizing antibody.
Abscissa: days after vaccination; ordinate: ymphocyte stimulation index.

Studies on the dynamics of PHA mitogen activity have shown that the amount of lymphocytes obtained from vaccinated children and transformed to blasts in culture was significantly decreased at various intervals. Maximal decrease in lymphocyte reactivity occurred on day 20 after the first vaccination. So, if the lymphocyte SI had been 437.08 ± 77.88 prior to vaccination, then by time of third vaccination (20th day) it decreased to 112.56 ± 16.82. The lymphocyte reactivity had been restored 50 days after the first vaccination, when the SI increased accordingly to 428.45 ± 97.32. No reduction in lymphocyte reactivity was observed for 50 days in control children who received placebo (Table 2).

Identification of T, B and nil lymphocytes

After vaccination an authentic reduction of all three lymphocyte populations was observed; a reduction in the amount of nil lymphocytes was registered within the first 10 days and subsequently their amount began reaching normal levels (1628.9 ± 52.4; 1255.8 ± 68.6; 1344.1 ± 51.7, respectively), while quantitative changes were more pronounced in the other two subpopulations. The amount of B lymphocytes which play the major role in the formation of specific humoral immunity had decreased by day 20 — as compared to the prevaccination amount — from 431.3 ± 13.9 to 291.4 ± 11.2 and reached the initial levels again by day 50 (416.6 ± 14.8). Quantitative changes in the subpopulation of T lymphocytes had been registered up to day 30; during this period their amount had decreased from 1556.3 ± 50.1 to 924.9 ± 53.9 and after 20 days it reached the initial levels (1415.1 ± 50.1) (Table 3).

Formation of humoral immunity

In this set of experiments the dynamics of serum antibody levels was analyzed in vaccinated children. Table 4 shows the results of serological assays. Antihaemagglutination antibody was detected in 76.67% of vaccinees, and 4- to 32-fold rises in antibody titres were observed. Similar results were obtained by testing of the same sera by Nt which showed 4- to 64-fold rises in the titres of virus neutralizing antibodies in 73.33% vaccinees. Antineuraminidase antibodies were observed as early as during the first 10 days, reached maximum by day 20 and remained on that level throughout the observation period. Titres of antineuraminidase antibody rose 4- to 32-fold in 83.33% vaccinees. No rises in the amount of antihaemagglutination, neutralizing and antineuraminidase antibodies were observed in sera of control children.

Analysis of the data obtained in BTA and Nt

Comparative studies of the indices of lymphocyte stimulation in vaccinated children revealed a small group of persons with an interesting regularity. In 8 out of 30 children who exhibited no rises in neutralizing antibody titres, indices of cellular immunity remained at a very low level. The SI in this group had increased from 1.68 $\pm$ 0.32 to 2.39 $\pm$ 0.40 by day 20, but at the end of the observation period (day 50) it was reduced to 1.82 $\pm$ 0.42. At the same time BTA indices were high in 22 vaccinated children with a 4-fold and higher rise in neutralizing antibody titres (Fig. 1).

Sensibilizing properties of the vaccine

Our investigations aimed to study the sensibilizing effect of the live influenza vaccine on a human organism have shown that the decrease in the amount of basophils and eosinophils was functional and remained within the standard range.

The next stage in our experiments was the study of the NII phenomenon which characterizes the degree of sensibilization of the organism. The results obtained have shown that the average value of NII was 0.018 $\pm$ 0.005 in the vaccinated group while in the control group it was 0.021 $\pm$ 0.007; then the NII began to increase gradually in the vaccinated group and reached maximum by day 20 (0.082 $\pm$ 0.01), but 50 days after the first vaccination it decreased in the vaccinated group practically to the initial level — 0.03 $\pm$ $\pm$ 0.005. The NII value did not exceed the upper limits of a physiological standard throughout the investigations (up to 0.08). The average value of NII was invariable in the control group.

Discussion

Bearing in mind the importance of studies on the effect of oral influenza vaccine (produced in the Moscow Research Institute for Viral Preparations) on human organism by immunological assays, we attempted to characterize the status of cell mediated immunity of the organism in the process of post-vaccination immunity. Our studies have shown that manifestation of post-

vaccination influenza immunity is associated with both the formation of serum antibodies and development of the cellular immunity. The increase of the index of lymphocyte stimulation from 1.81 ± 0.14 to 11.87 ± 2.83 by the end of the 3rd week after the first vaccination indicates that a gradual sensibilization of immunocompetent lymphocytes to influenza virus occurs in the organism, and it was observed even 50 days after the beginning of vaccination. It was demonstrated that the children who showed no rises in the amount of virus neutralizing antibodies, had very low indices of BTA. The non-specific (PHA mitogen) BTA showed that vaccination of children resulted in a decrease of lymphocyte reactivity within the first 20 days, but in 50 days after the beginning of vaccination, the lymphocyte reactivity had been restored. A decrease in the amount of T lymphocytes in the subpopulation within 1 month after vaccination apparently indicated impairment of immunocompetent T cells with influenza virus. The results obtained are in good agreement with the data of other workers (Rudenko *et al.*, 1978; Kimsey *et al.*, 1979) who demonstrated marked physiological and immunological changes in levels of T lymphocytes in the course of viral infections and a short immunodepression in persons vaccinated with a live influenza vaccine. The data obtained in HI, Nt and NI tests testify to efficient stimulation of a specific humoral response. Later on, studies of specific sensibilization of the organism with viral preparations and estimation of changes associated with the intensity of vaccination reactions have led to determinatin of the reactivity of blood cells which are known to play a role in delayed and immediate type allergic reactions.

The sensibilization index (NII test) which had been determined in three times vaccinated children, was within the limits of physiological standards and correlated with the blood cell counts. Therefore, it has been ascertained that live tissue influenza vaccine has poor sensibilizing properties. More thorough studies of immunological status in human trials of different influenza vaccines can contribute to their safety for humans.

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