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BLASTOGENIC LYMPHOCYTE RESPONSE AS INDICATOR OF CELL-MEDIATED IMMUNITY IN HUMANS VACCINATED WITH LIVE AND INACTIVATED INFLUENZA VACCINES

L. G. KARPOVICH, T. V. KALASHNIKOVA, S. G. DZAGUROV, V. V. BLOKHA, V. L. ROMANOV, Yu. R. BAKEEV, Z. B. ITKIN, N. I. LONSKAYA

The L. A. Tarasevich State Research Institute of Standardization and Control of Medical Biologic Preparations of the U.S.S.R. Ministry of Public Health, 121002 Moscow, U.S.S.R.

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Summary. — The level and dynamics of lymphocyte blastogenesis in response to phytohaemagglutinin (PHA) and to specific influenza virus antigen were studied in 3 groups of humans, vaccinated with live or inactivated whole virion influenza vaccines (H3N2 type) and placebo (control group). Both live and inactivated influenza vaccines did not change significantly the functional activity of T lymphocytes as determined by the mean values of stimulation index (SI). The analysis of individual values of PHA-dependent blastogenic response, however, revealed a decrease in SI as compared with its prevaccination level in $33.3 \pm 11.4\%$ of the vaccinees given the live influenza vaccine.

Key words: influenza A virus; lymphocyte blastogenic response; stimulation index

Introduction

The immune response and its role in antiviral immunity has been recently studied rather intensively. Nevertheless, the role of some components of cell-mediated immunity (CMI) in developing resistance to influenza virus has not been fully cleared, although results indicate the protective role of CMI (Bardina *et al.*, 1979; Ennis *et al.*, 1974; Yap and Ada, 1978). Therefore, it seemed of special interest to study CMI in humans and experimental animals vaccinated with different types of viral vacines in order to define the protection mechanisms post-vaccination and to improve the specific prophylaxis.

In the present work, CMI was followed by lymphocyte blastogenic assay (LBA) in comparison with the serologic response in humans vaccinated with a commercial live and an inactivated (whole virion) influenza vaccines. The study included: 1) determination of the level of the so-called spontaneous blastogenesis (without in vitro stimulation of cells with any mitogen) of lymphocytes in groups of immunized and control subjects; 2) the dynamics and level of T lymphocyte blastogenic response in the same subjects after in vitro stimulation with phytohaemagglutinin (PHA) as a polyclonal, mostly T lymphocyte — activator;3) evaluation of the level and dynamics of LBA in the vaccinees after in vitro stimulation with influenza virus antigen; 4)

comparison of the indices of antigen-dependent lymphocyte proliferation in LBA with the results of serologic tests.

Materials and Methods

Vaccinees. The contingent of vaccinees included subjects in the age from 18 to 25 years, with similar occuaptions and living conditions. Three groups, 20 persons each, formed by random selection, included 2 experimental and 1 control group.

Vaccines. A commercial live influenza vaccine from the A/Victoria/3/75 strain (H3N2) manufactured by Stavropol Institute of Vaccines and Sera, and an inactivated whole virion vaccine prepared at Pasteur Institute of Epidemiology and Microbiology, Leningrad, from the A/ /Texas/1/77 (H3N2) strain by chromatography on macroporous glass were used.

Vaccination schedules. According to the instruction for use, the live influenza vaccine was given intranasally in a volume of 0.5 ml twice at a month apart. The inactivated chromatographic vaccine was inoculated intramuscularly once in a 0.2 ml amount. Control subjects received placebo (apyrogenic buffered physiological solution) as follows: one half intranasally — 2 doses of 0,5 ml a month apart — and the second half a single dose of 0.2 ml intramuscularly.

Examinations of the vaccinees. Specimens were collected from the vaccinees and control subjects imediately before immunization, on days 8 and 22 after vaccination, and on day 8 after revaccination, i.e. 35 days after primary vaccination with the live influenza vaccine, respectively.

Influenza virus antigens. Influenza virus strains A/Texas/1/77 (H3N2) and A/Victoria/3/75 (H3N2) were concentrated and purified according to the method of Smirnov et al. (1981). The viruses were inactivated by UV irradiation (PRK-2 lamp) for 4 min from a distance of 10 cm. The degree of inactivation was checked by inoculation into the allantoic cavity of 11-day-old chick embryos followed by tests on the allantoic fluid in the haemagglutination test (HA). The control antigen consisted of allantoic fluid of uninfected chick embryos purified and diluted as the virus antigen.

LBA was performed according to Aurich *et al.* (1979) using PHA and the influenza virus antigens. PHA (M, Difco) was added in the dose of 20 μg per 10^6 cells. ^{3}H-thymidine (specific activity 543 GBq/mmol) was obtained from Department of VO Isotope, Leningrad. Lymphocytes were recovered from heparinized blood plasma (25 units of heparin "Spofa" per 1 ml) by treatment with sterile gelatin according to Braude and Goldman (1967). 200 haemagglutination units of antigen were added per 10^6 cells (Kalashnikova *et al.*, 1981).

Leukocytes washed twice with medium 199 were cultivated at 37 °C on the bottom of penicillin vials in amounts of 1×10^6 cells in 2 ml of medium 199 containing antibiotics (100 units of penicillin and 50 μg streptomycin per 1 ml) and 10% inactivated human serum from the blood of group IV (AB) showing < 10 HI antibody titre to influenza (H3N2) virus.

PHA ("M" Difco in a dose of 20 μg), the viral antigen (200 HA units) and control antigens were added in 0.1 ml volumes per vial with 10^6 cells.

^{3}H-thymidine (185 kBq per specimen, in a volume of 0.025 ml) was added to the culture of PHA-stimulated lymphocytes at 48 hr and into the specimens with influenza virus antigen at 120 hours of cultivation. In 24 hr after the addition of the label, the cells were placed on "Synpore" filters (Praha) with a pore diameter of 0.85 μm, washed successively with physiological saline solution and 5% trichloracetic acid, fixed with ethanol, and the label incorporated into proliferating cells was couted in the scintillation couter "Intertechnique" (France). The reaction was assessed by the SI of blastogenesis which was a ratio of the number of c.p.m. in lymphocyte cultures stimulated with PHA or antigen to the number of c.p.m. in control (non-stimulated) cells. The results were analysed statistically by the Student's t-test. The validity of association between the values was calculated by the method of rank correlation.

The HI test was performed by the conventional method.

Results

Examination of lymphocytes recovered from peripheral blood of the subjects vaccinated with the live or the inactivated (chromatographic) influenza (H3N2) vaccine and of the control subjects showed no significant changes of spontaneous blastogenesis.

Table 1. Lymphocyte blastogenic response to PHA in subjects vaccinated with liver or inactivated influenza vaccines (M ± m)

Vaccine	Postvaccination interval (days)			
	0*	8	22	35
Live	77.8 ± 9.7	85.1 ± 11.3	60.6 ± 9.5	89.5 ± 8.9
Inactivated	73.8 ± 8.3	70.7 ± 8.7	71.1 ± 5.9	—
Control	62.0 ± 5.2	73.1 ± 7.4	70.2 ± 11.6	69.8 ± 6.2

* Prevaccination examination

Similarly, the blastogenic response of lymphocytes stimulated in vitro with PHA (Table 1) did not significantly change at any interval of examination as evidenced by mean stimulation indices in each group. However, the analysis of individual values of T-dependent lymphocyte blastogenesis revealed a significant ($P < 0.05$) decrease in lymphocyte SI on day 22 in 6 subjects (33.3 ± 11.4%) immunized with live influenza vaccine as compared with the prevaccination levels. In two of them low values of PHA-dependent lymphocyte blastogenesis were found also on day 35 (the 8th day after revaccination). In the group given inactivated influenza vaccine only one subject had a low blastogenic response to PHA on days 8 and 22 post-vaccination. No subjects with reduced blastogenic activity of T lymphocytes were found in the control group.

Of special interest was the analysis of lymphocyte blastogenic response to stimulation with influenza virus antigen in vitro in immunized and control

Table 2. The dynamics of LBA from immunized and control subjects in response to influenza virus antigen stimulation in vitro (M ± m)

Vaccine	Virus antigen	Postvaccination interval (days)			
		0	8	22	35
Live	A/Victoria/ 3/75	22.7 ± 10.8* / 1.21 ± 0.12	75.0 ± 11.1 / 1.96 ± 0.23	26.6 ± 11.8 / 1.15 ± 0.11	38.8 ± 11.8 / 1.33 ± 0.11
Inactivated	A/Texas/ 1/77a	12.5 ± 8.5 / 1.01 ± 0.09	55.0 ± 11.4 / 1.54 ± 0.12	12.5 ± 8.5 / 1.14 ± 0.08	—
	A/Victoria/ 3/75	16.6 ± 9.0 / 1.15 ± 0.07	28.5 ± 1.5 / 1.20 ± 0.09	0 / 1.02 ± 0.06	8.3 ± 8.3 / 1.08 ± 0.06
Control	A/Texas/ 1/77a	11.1 ± 7.6 / 1.02 ± 0.03	26.6 ± 11.8 / 1.16 ± 0.07	11.1 ± 9.8 / 1.00 ± 0.11	—

*) Numerator — per cent of subjects whose lymphocytes responded positively to influenza virus antigens (SI ≧ 1.5); denominator — arithmetic mean of SI ± arithmetic mean error in the groups examined.

Table 3. The dynamics of HI antibody response in vaccinees given live and inactivated influenza vaccines

Vaccine (group of vaccinees)	Virus antigen used	Postvaccination interval (days)			
		0	8	22	35
Live	A/Victoria/ 3/75	56.5 ± 1.2*	58.5 ± 1.2	79.5 ± 1.2	85.9 ± 1.2
Inactivated	A/Texas/ 1/77a	47.5 ± 1.2	154.8 ± 1.3	301.3 ± 1.2	—
	A/Victoria/ 3/75	53.4 ± 1.2	54.3 ± 1.2	54.3 ± 1.2	52.4 ± 1.3
Control	A/Texas/ 1/77a	49.3 ± 1.2	52.3 ± 1.2	51.4 ± 1.2	52.3 ± 1.3

*) Reciprocal geometric mean antibody titres as determined by HI test (M ± m)

subjects. Before vaccination, the proportion of subjects whose lymphocytes had been sensitized to influenza virus and responded in vitro by antigen--dependent proliferation did not exceed 27.7% in any of 3 groups under study (Table 2). On day 8 post-vaccination this proportion increased significantly to 55% in the subjects immunized with the inactivated and to 75% in those immunized with live influenza vaccine ($P < 0.05$). Subsequently, the proportion of vaccinees with positive blastogenic response to influenza virus antigen decreased to 20.0—26.6%. In the control group, the proportion of the subjects whose lymphocytes showed increased blastogenesis in response to stimulation with influenza virus antigen in vitro did not exceed 28.5% throughout the observation period.

Analysis of the mean values of SI of lymphocytes in each group revealed a significant ($P < 0.05$) increase in lymphocyte blastogenesis by the influenza virus antigen in vaccinees given either inactivated or live influenza vaccine, on day 8 post-vaccination as compared with the corresponding level before vaccination or in the control group at a similar interval (Table 2). On day 22, the blastogenic response of lymphocytes to virus antigen decreased in the groups of vaccinees to the initial prevaccination levels. No significant increase in the antigen-dependent blastogenesis was observed on day 8 after

Table 4. Proportion of seroconversions in the subjects vaccinated with live and inactivated influenza vaccines (M ± m)

Vaccine	Virus antigen used in HI test	Post-vaccination interval (days)		
		8	22	35
Live	A/Victoria/3/75	5.0 ± 5.0	20.0 ± 3.6	34.5 ± 10.0
Inactivated	A/Texas/1/77	36.8 ± 1.4	90.0 ± 6.9	—
Control	A/Victoria/3/75	5.2 ± 5.2	10.5 ± 7.2	5.0 ± 5.0
	A/Texas/1/77	10.5 ± 7.2	5.2 ± 5.2	—

revaccination (day 35 post primary vaccination) in the group given live influenza vaccine.

In the control group, mean SI in response to influenza virus antigen did not change significantly throughout the observation period being in the range from 1 to 1.2.

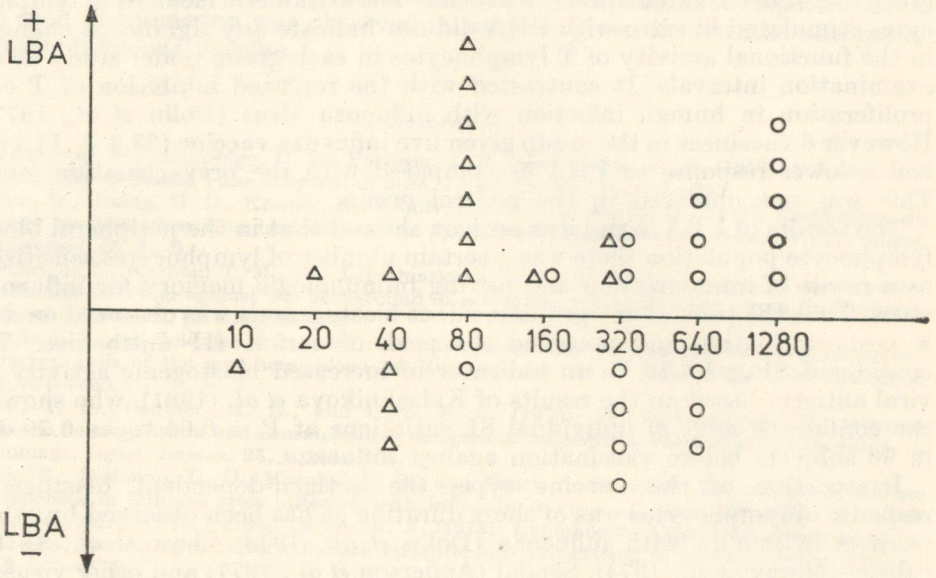

Fig. 1.

Comparison of HI antibody titres and LBA in subjects vaccinated with live (△) or inactivated (○) influenza vaccines.

Abscissa: antibody titres (serum dilution reciprocals) on day 22 after vaccination; ordinate: positive (SI ≧ 1.5) and negative (SI < 1.5) LBA in vaccinees on day 8 post vaccination. △ = live vaccine; ○ = inactivated whole virion vaccine.

The humoral response as determined by the presence of HI antibodies was found by day 22 post-vaccination (Tables 3 and 4). The proportion of seroconversion in the group given inactivated chromatographic influenza vaccine was 90%. In those given live vaccine it was 20% on day 22 post vaccination and 31.5% on day 8 after revaccination, respectively. Reciprocal geometric mean titres of HI antibodies by the 22nd day were 301.3 ± 1.2 in the former and only 79.5 ± 1.2 in the latter. When the antigen-dependent lymphocyte proliferation was compared with serological values individually for each vaccinee in the groups under study, the subjects whose lymphocytes had increased blastogenic response to influenza virus antigen stimulation in vitro (SI ≧ 1.5) on day 8 post-vaccination were found to have also higher antibody titres to influenza virus on day 22 by HI test (see Fig. 1). A significant direct correlation ($P < 0.05$) was established between these values in each group under study.

Discussion

The above study of CMI by one of its responses, i.e. by lymphocyte blastogenesis stimulated with: PHA and specific influenza virus antigen in vitro, allowed more detailed follow-up of the dynamics of CMI response in vaccinees given live or inactivated influenza vaccine. The arithmetic mean SI of lymphocytes stimulated in vitro with PHA did not indicate any significant changes in the functional activity of T lymphocytes in each group under study at all examination intervals. It contrasted with the reported inhibition of T cell proliferation in human infection with influenza virus (Dolin *et al.*, 1977). However 6 vaccinees in the group given live influenza vaccine (33.3 $\pm$ 11.4%) had a lower response to PHA as compared with the prevaccination level. This was not observed in the control group.

The results of LBA with virus antigen showed that in the peripheral blood lymphocyte population there was a certain number of lymphocytes sensitized as a result of immunization and having immunologic memory for influenza virus. Thus the peak of antigen-dependent blastogenesis was observed on day 8 post-vaccination and preceded the peak of serum HI antibodies. We considered SI $\geqq$ 1.5 to be an indicator of increased blastogenic activity to viral antigen, based on the results of Kalashnikova *et al.* (1981), who showed the confidence limit of individual SI variations at P = 0.05 to be 0.7—1.5 in 96 subjects before vaccination against influenza.

Irrespective of the vaccine type, the antigen-dependent blastogenic response of lymphocytes was of short duration as has been observed by other workers in studies with influenza (Dolin *et al.*, 1978; Chow *et al.*, 1979), rubella (Moray *et al.*, 1974), Sendai (Anderson *et al.*, 1977) and other viruses. Revaccination with live influenza vaccine did not produce a secondary enhancement in lymphocyte blastogenesis in response to stimulation with virus antigen in vitro. This may be 1. due to the formation by this time of a clone of B lymphocytes capable of responding to subsequent antigen stimulation by antibody production which, according to some authors (Jacobs *et al.*, 1972; Oppenheim, 1972), could inhibit the antigen-stimulated proliferation of sensitized lymphocytes or 2. due to the decrease (observed in this study) in the functional activity of T lymphocytes (apparently helpers) which could also inhibit blastogenic response of B lymphocytes.

The results of LBA with mitogens and specific antigens carried out at various intervals after vaccination, alongside with other cell assays and serologic tests demonstrate not only the immunogenic potency but also immunologic innocuity of vaccine preparations defined by some authors (Semenov and Gavrilov, 1976; Kraskina, 1979; Peradze and Fridman, 1979) as retention of the immune system homeostasis after vaccination.

The antigen-dependent proliferation of lymphocytes indicating the appearance of a clone of sensitized lymphocytes in subjects immunized with live and inactivated influenza vaccines as well as subsequent development and rise of antibodies to influenza virus attest to the immunogenic potency of the vaccine preparations under study.

At the same time, a decreased lymphocyte blastogenic response to PHA

stimulation in vitro observed on day 22 post-vaccination in 33.3 ± 11.4% of the subjects given live influenza vaccine and subsequent low antigen-dependent lymphocyte blast transformation on day 8 after revaccination together with low serologic titres as compared to those in the group given inactivated chromatographic vaccine may indicate some suppressive effect of the live vaccine on the functional activity of immunocompetent cells. No such phenomenon was observed in subjects given inactivated chromatographic influenza vaccine.

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