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IMMUNE RESPONSE TO DIFFERENT TICK-BORNE ENCEPHALITIS VACCINES AS REVEALED BY LYMPHOCYTE BLAST TRANSFORMATION AND VIRUS NEUTRALIZATION TESTS

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Summary. — Immunological memory has been demonstrated in lymphocytes of persons vaccinated against tick-borne encephalitis (TBE). Stimulation indices (SI) of lymphocytes in response to TBE virus antigens in lymphocyte blast transformation test increased after complete vaccination course with the commercial vaccine; by this time, sera of vaccinees contained virus neutralizing antibodies. The concentrated purified vaccine had a higher immunogenic activity, as evidenced by increased SI and virus neutralizing antibodies (NA) in the vaccinated subjects already after the first and second vaccinations.

Key words: tick-borne encephalitis; concentrated vaccine; blast transformation; virus neutralization test

Introduction

Clinical findings (Mayer *et al.*, 1976; Kvetkova *et al.*, 1978*a*, *b* 1980; Pervikov *et al.*, 1981) and experimental data (Bukovskaya *et al.*, 1978; Khozinsky, Semenov, 1980; Rabinowitz, 1976*a*, *b* and others) indicate to the importance of cellular mechanisms of immune response in resistance to arbovirus infections. So far, investigations on cellular immunity have been scarcely involved either in the study of immunogenicity and reactogenicity of the commonly used tick-borne encephalitis (TBE) vaccine or in the efficacy of newly developed ones.

Our earlier data on postvaccinal immunity against TBE in vaccinated humans (Vorobieva *et al.*, 1979; Shalamberidze, 1981) suggested that inactivated TE vaccine could affect the development of cellular immunity and that the latter had correlated with the humoral immune response. The goal of the present work has been to study the dynamics of antigen-dependent blast transformation (BT) of lymphocytes in comparison with the dynamics of humoral immunity (virus NA) after administration of different TBE

vaccines. The investigations have been carried out in the framework of epidemiological assessment of vaccines aiming at possible introducing of a concentrated vaccine into practical use.

Materials and Methods

Vaccinated persons: all 74 volunteers were males in the age of 180—20 years.

TBE virus strain "Sofyin" was isolated in mice in 1937 in the Primorye Territory from the brain of a patient who died of encephalitis. This strain represents the eastern antigenic variant of the virus, its neutralization index with homologous immune serum was 3.0 log LD_{50}. Stock virus titre in outbred albino mice after intracerebral (i.c.) inoculation was 9.44 $\pm$ 0.57 log LD_{50}/ml, after intraperitoneal inoculation 7.16 $\pm$ 0.23 log LD_{50}/ml and subsutaneous inoculation 6.54 $\pm$ $\pm$ 0.24 log LD_{50}/ml, respectively (the invasivity index — 2.94 $\pm$ 0.26).

Yellow fever virus, strain Dakar was obtained from the Institute of Poliomyelitis and Viral Encephalitides (IPVE), U.S.S.R. Academy of Medical Sciences, Moscow. Its neutralization index with homologous immune serum was 3.1 log LD_{50}. The stock titre in outbred albino mice after i.c. infection was 6.7—7.0 log LD_{50}/ml.

TBE vaccines. 1. Commercial tissue culture inactivated sorbed liquid vaccine manufactured at IPVE, U.S.S.R. Academy of Medical Sciences, Moscow, lot No. 897, control number 189 (immunogenicity index 7.0 log LD_{50}; residual formalin — 0.026% sorbent Al_2O_3 — 0.13%, total protein — 418 μg/ml). This vaccine is the inactivated antigen of TBE virus, sorbed on aluminium hydroxide harvested in chick embryo cells. 2. Tissue culture inactivated dried vaccine concentrated and purified by continuous zonal buoyant density ultracentrifugation, manufactured at IPVE, U.S.S.R. Academy of Medical Sciences, Moscow, series 9 (immunogenicity index — 8.0 log LD_{50}; MID_{50} — 0.004 ml; residual formalin — 0.015%, total protein — 175 μg/ml).

Both vaccines were prepared from the commercial TBE virus strain "Sofyin".

Vaccination schedules. Schedule 1: in accordance with the instruction for use of commercial TBE vaccine (3 doses with 7 and 14 day intervals, the fourth — 6 months from the onset of vaccination). Schedule 2: two doses with a 30 day interval, the third — 6 months from the onset of vaccination. The vaccines (1.0 ml volumes) were injected subcutaneously.

Intervals of examination: 1st — before vaccination, 2nd — on day 7 after the first dose, 3rd — on day 30 after the first vaccine dose, 4th — on day 7 after the second vaccine dose, 5th — before the last vaccine dose, 6th — on day 7 after the last vaccine dose. At these intervals venous blood was drawn from vaccinated persons.

Design of the BT test. Radioisotopic method was used (Veskova *et al.*, 1977) without leukocyte separation. Leukocytes were incubated at 37 °C for 144 hr (1×10^6 cells in 1.0 ml of medium 199 supplemented with 10% human group IV blood serum). Before incubation, viral antigens at a proportion of 50 μl/ml were placed into test tubes, the controls received 50 μl of medium 199. At 18 hr before the end of incubation, 188.6 kBq/ml of ^{3}H-thymidine was added into the tubes. After incubation, the cells were precipitated onto nitrocellulose filters "Synpor" (Czechoslovakia) with pore diameter of 0.85 μm, after that the filters were successively washed in isotonic solution of sodium chloride (twice), 5% cooled (4 °C) trichloraceric acid solution and ethanol. The filters were throroughly dried and their radioactivity was counted in liquid scintillation counter "Intertechnique" (France). The results were expressed in stimulation indices (SI) — the ratio of the c.p.m. in the sample to the c.p.m. in controls.

Lot No. 9 of concentrated purified vaccine diluted 1 : 5 and dialyzed for 24 hr against medium 199 served as TBE virus antigen. Control antigen (strain "Dakar" of yellow fever virus) was prepared from a virus-containing suspension of mouse brain, purified and concentrated up to 10^9 i.c. LD_{50}/ml by supercentrifugation and inactivated by ultraviolet irradiation (PRK-7 lamp) at 15 cm distance for 4 min.

^{3}H-thymidine of Leningrad division of the V/0 "Isotop" with specific activity of 7.6 GBq/mmol was used.

Virus NA in sera of vaccinated volunteers were titrated using conventional technique of neutralization in mice inoculated by i.c. route. The TBE virus strain "Sofyin" homologous to the vaccine strain was used. Sera were regarded as positive if their neutralization index was 1.7 log LD_{50} and higher. The results were statistically treated according to Fischer (Urbakh, 1964).

Table 1. Titration of inactivated antigen of TBE virus in lymphocyte BT test

Schedule	Volunteers examined	SI of lymphocytes in BT with the antigen* at dilutions				
		none	1 : 2	1 : 4	1 : 8	1 : 16
Vaccinated twice with the concentrated vaccine	M. I.	1.10	1.07	1.58	1.83	1.12
	V. N.	1.00	1.21	1.62	1.54	0.74
	Sh. P.	1.37	1.17	1.51	2.01	1.21
	B. S.	n.t. **	0.87	1.62	0.87	n.t.
	N.	n.t.	1.00	1.01	1.34	1.14
Non-vaccinated	Z. N.	0.99	0.93	0.88	1.27	0.67
	O. N.	0.97	0.84	n.t.	0.91	1.09
	K. S.	1.05	n.t.	0.91	0.93	1.01

Notice: * the series No. 9 of concentrated purified TE vaccine was used as antigen.
** n.t. — not tested.

For qualitative scoring of lymphocyte SI by antigens, confidence limits of this index in non-vaccinated individuals at 95% significance were determined by the formula $M \pm 2\sigma$. All individual SI above the upper confidence limit (in our experiments it was 1.5) were regarded as positive. To determine the significance of differences in the incidence of the parameter tested in the two groups, the precise Fischer technique for four-entry table was used (Genes, 1964). The differences were considered statistically significant at $P < 0.025$.

Results

BT of lymphocytes

The results of preliminary titration in lymphocyte BT of the antigen prepared from concentrated vaccine lot No. 9 (Table 1) has shown that it was the most active at 1 : 4—1 : 8 dilutions with lymphocytes of subjects vaccinated twice with concentrated vaccine. At these dilutions, lymphocytes of non-vaccinated subjects were not stimulated in BT test. Based on these results the antigen was further used at 1 : 5 dilution.

The analysis of BT test in response to TBE virus antigen *in vitro* has demonstrated an elevation of mean SI of lymphocytes in subjects inoculated with the employed TBE vaccine (Table 2). As a rule, the administration of commercial vaccine caused an elevation of mean SI only after complete vaccination course, whereas the concentrated purified vaccine induced SI elevation after the first and the second vaccinations, respectively. A significant increase in the number of subjects with positive SI of lymphocytes in response to TBE virus antigens was observed with the commercial vaccine after completion of the vaccination schedule only (Table 3). Among the subjects vaccinated with concentrated vaccine this parameter increased significantly already after the first and the second vaccinations. When the control antigen from strain Dakar of yellow fever was used in BT test, no elevation either of the mean SI or of the number of positively responding subjects during vaccination was observed.

It should also be noted that among volunteers inoculated with the commercial

Table 2. Mean lymphocyte SI in response to TBE virus antigen in vaccinated subjects (M ± m)

Examination	Vaccine			
	commercial		concentrated	control group
	schedule No. 1*	schedule No. 2*	schedule No. 2	schedule No. 2
1st	1.09 ± 0.04	1.01 ± 0.06	1.11 ± 0.07	1.02 ± 0.02
2nd	0.96 ± 0.06	1.24 ± 0.10	1.47 ± 0.11	0.93 ± 0.06
P	> 0.05	> 0.05	< 0.05	> 0.05
3rd	1.03 ± 0.07	1.13 ± 0.11	1.01 ± 0.07	1.04 ± 0.06
P	> 0.05	> 0.05	> 0.05	> 0.05
4th	n.t.	1.17 ± 0.16	1.44 ± 0.09	1.15 ± 0.10
P		> 0.05	< 0.01	> 0.05
5th	1.08 ± 0.08	1.12 ± 0.06	1.13 ± 0.05	1.09 ± 0.08
P	> 0.05	> 0.05	> 0.05	> 0.05
6th	1.41 ± 0.11	1.41 ± 0.07	1.88 ± 0.23	1.10 ± 0.05
P	> 0.02	> 0.001	< 0.01	> 0.05

* for vaccination schedules see Materials and Methods.

Table 3. The number of volunteers with positive lymphocyte SI (≧ 1.5) in lymphocyte BT tests in response to TBE virus antigen

Examination	Vaccines		
	commercial		concentrated
	schedule No. 1	schedule No. 2	schedule No. 2
1st	0/16	0/16	1/15
2nd	0/17	0/17	11/17
P	> 0.025	> 0.025	< 0.01
3rd		1/9	0/13
P	n.t.	> 0.025	> 0.025
4th		1/6	7/9
P	n.t.	> 0.025	< 0.01
5th	0/10	1/14	0/12
P	> 0.025	> 0.025	> 0.025
6th	6/12	6/12	5/7
P	< 0.01	< 0.01	< 0.01

Notice: numerator — number of subjects with positive reaction; denominator — number of subjects examined. Further explanations in Table 2.

Table 4. Detection of NA in the sera of volunteers vaccinated with different TBE vaccines

Examination	Vaccine		
	commercial		concentrated
	schedule No. 1	schedule No. 2	schedule No. 2
1st	0/10	0/8	0/9
2nd	0/10	1/8	1/9
P	> 0.025	> 0.025	> 0.025
3rd	n.t.	0/8	5/9
P		> 0.025	< 0.025
4th	n.t.	2/8	4/8
P		> 0.025	> 0.025
5th	3/10	2/8	9/9
P	> 0.025	> 0.025	< 0.01
6th	4/10	4/8	9/9
P	> 0.025	> 0.025	< 0.01

vaccine, no significant difference between SI in the groups of subjects vaccinated according to the two different schedules was found.

The sera of inoculated subjects were assayed in neutralization test using TBE virus strain Sofyin (Table 4). It has been shown, that the number of subjects whose sera contained NA with neutralization index 1.7 and higher, was confidently greater before the last vaccination in the group vaccinated with the concentrated vaccine than among those inoculated with the commercial vaccine, independently of the administration schedule ($P < 0.01$). On day 7 after the last vaccination, the percentage of those whose blood contained NA was higher among the subjects inoculated with concentrated vaccine than among those treated with the commercial vaccine according to the schedule No. 1 ($P < 0.01$).

Discussion

It has been found that in vaccinated subjects the level of virus-specific BT of lymphocytes increased, the dynamics of this increase being different in the group of commercial vaccination and the group receiving the concentrated TBE vaccine. A significant increase of the mean SI of lymphocytes in response to the antigen was observed in volunteers vaccinated with the commercial vaccine by schedules No. 2 (from 0.01 ± 0.06 up to 1.41 ± 0.07; $P < 0.001$) and No. 1 (from 1.09 ± 0.04 to 1.41 ± 0.11; $P < 0.02$) after a complete vaccination course (3—4 doses). Among vaccinees who were given the concentrated vaccine, the index was significantly higher than the initial one (1.11 ± 0.07) after the first (1.44 ± 0.11; $P < 0.05$), also after the second (1.44 ± 0.09; $P < 0.01$) as well as after the last (1.88 ± 0.23; $P < 0.01$) vaccinations.

The same regularities have been found by the analysis of individual SI of lymphocytes. After the first vaccination with concentrated vaccine, in 11 out of 17 subjects positive SI (≥ 1.5) have been registered in response to the antigen while after the second vaccination this was true in 7 out of 9 vaccinees. In both cases, the values were higher than in the subjects inoculated with commercial vaccine tested at the same time. After the complete vaccination course in 5 out of 7 subjects immunized with the concentrated vaccine positive stimulation indices were observed, while after the commercial vaccine this was true only in the half of them. No response of lymphocyte BT of vaccinated subjects to control antigen of yellow fever virus has been observed. It was demonstrated that the concentrated purified TBE vaccine prepared in IPVE, Moscow, had better immunogenic properties than the commercial vaccine. Studies of virus-specific BT of lymphocytes of subjects inoculated with TBE vaccine are very scarce. Glotov *et al.* (1980) report the increase in mean SI of lymphocytes to viral antigen from 1.8 ± 0.009 up to 2.4 ± 0.3 after 3 vaccinations with the commercial vaccine.

Elbert *et al.* (1981), Pervikov *et al.* (1981*a*, *b*) compared the SI of lymphocytes in subjects after vaccination with commercial and concentrated TBE vaccines. Using different vaccination schedules and different BT modifications, the authors obtained similar data. It has been also pointed out, that the method of lymphocyte BT is more sensitive than the reaction of lymphocyte migration inhibition for the assessment of cellular immunity in vaccinated subjects.

Our findings, consistent with the data of literature also suggest that lymphocyte BT allows to differentiate between vaccines with different concentrations of viral antigen in respect to their ability to induce the formation of specific cellular immunity in vaccinated subjects.

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