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VIRUS-INDUCED BLAST TRANSFORMATION OF LYMPHOCYTES AND ITS INHIBITION BY $F(ab^1)_2$ FRAGMENTS OF HUMAN IgG

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Summary. — Blast transformation of lymphocytes from persons immunized by vaccines against tick-borne Japanese encephalitides, in response to stimulation by homologous viral antigens was studied. ^{3}H-Thymidine incorporation into lymphocytes was completely inhibited by $F(ab^1)_2$ fragments of normal human IgG containing no antibody to the viruses examined. A correlation of the inhibitory action of $F(ab^1)_2$ fragments on lymphocyte transformation induced by viruses and phytohaemagglutinin was observed.

Key words: blast transformation; Flavivirus; phytohaemagglutinin; immunoglobulin

Introduction

Blast transformation of lymphocytes by phytomitogens or specific antigens has been used to evaluate the activity of lymphocytes both under normal conditions and those of antigenic stimulation (Cerber *et al.*, 1977; Baranova *et al.*, 1978). This reaction has also been employed to evaluate the state of immunity of man and animals in viral infections (El Araby *et al.*, 1978; Rasmussen and Merigan, 1978; Whittle *et al.*, 1978).

The intensity of the blast transformation reaction can be affected by numerous factors. In particular, it was found that Fab fragments of normal IgG, formed on proteinase digestion of IgG, are able to inhibit phytomitogen-induced lymphocyte transformation (Kulberg *et al.*, 1977). In this connection it was of interest to show whether Fab fragments may affect the regulation of antigen-induced blast transformation of lymphocytes.

In the present study we investigated the effect of vaccination with antiviral preparations on the antigen-dependent blast transformation of lymphocytes and the effect on this reaction of $F(ab^1)_2$ fragments of normal human IgG.

Materials and Methods

Human volunteers were immunized with (*1*) experimental inactivated concentrated and purified vaccine against tick-borne encephalitis (TBE) prepared by zonal centrifugation in a sucrose density gradient (Elbert *et al.*, 1980); and (*2*) commercial inactivated brain vaccine against Japanese encephalitis (JE) (Toshiba Kagamu Kogyo Co., Ltd., Japan). Lymphocytes from the donors' peripheral blood were obtained according to Boyum (1965). The blast transformation reaction was carried out and ^{3}H-thymidine incorporation into DNA was determined as described (Kulberg *et al.*, 1977). As stimulating antigen of TBE virus we used various volumes of infected culture fluid containing $10^{10}-10^{11}$ formalin-inactivated virus particles per ml. A formalin--inactivated 10% infected mouse brain suspension served as JE virus antigen. In control experiments, the lymphocytes were stimulated by antigens from uninfected brain tissue or cell cultures. In concentrations corresponding to those in working dilutions of the stimulating viral antigens the control antigens showed no blast-transforming activity.

The intensity of ^{3}H-thymidine incorporation into lymphocyte DNA was evaluated by the stimulation index (I_s):

$$I_s = \frac{\text{Mean radioactivity of cells stimulated by viral antigen}}{\text{Mean radioactivity of non-stimulated cells}}$$

$F(ab^1)_2$ fragments used in the experiments were obtained by pepsin treatment (Nisonoff *et al.*, 1960) of normal human IgG free of antibody to the viruses tested.

Results and Discussion

Preliminary experiments showed that lymphocyte blast transformation induced by viral antigens can be demonstrated by the respective viral preparations 4-14 days after immunization. An increased ^{3}H-thymidine incorporation into lymphocyte DNA was observed 4, 5 and 6 days after addition of viral antigen (Table 1). The effect was specific: cells from persons vaccinated against TBE showed no increased ^{3}H-thymidine incorporation after stimulation with JE virus and vice versa.

Starting with the threshold concentration, an increase in the amount of virus particles in the antigen caused no increase in the intensity of the reaction, which was in accordance with the results reported by Waner and Budnick (1977). After two and three immunizations with the preparations examined, positive reactions were obtained in 60—75% of the volunteers,

Table 1. Blast transformation of lymphocytes induced by TBE virus (^{3}H-thymidine incorporation in count/min with 95% confidence interval, M±m)

Exp. No.	Stimulating antigen*	Lymphocytes incubated for 4 days	5 days	6 days
1	None	385 ± 69	388 ± 80	350 ± 82
2	TBE, 0.01 ml	714 ± 161	675 ± 100	667 ± 63
		$P_{2-1} < 0.05$	$P_{2-1} < 0.05$	$P_{2-1} < 0.05$
3	TBE, 0.001 ml	757 ± 114	778 ± 195	447 ± 94
		$P_{3-1} < 0.05$	$P_{3-1} < 0.05$	$P_{3-1} > 0.05$
4	TBE, 0.0001 ml	298 ± 52	411 ± 97	402 ± 77
		$P_{4-1} > 0.05$	$P_{4-1} > 0.05$	$P_{4-1} > 0.05$

*The indicated volume of antigen per 2.5×10^5 lymphocytes. The Table presents the results of one of 5 experiments which yielded similar results. Lymphocytes were isolated 7 days after immunization.

Table 2. Inhibition of virus- and phytohaemagglutinin-(PHA-) induced blast transformation by $F(ab^1)_2$ fragments of human IgG (M±m)

Exp. No.	Source of lymphocytes	Stimulation	Lymphocytes cultivated for 3 days	5 days	I_s	P
1	TBE vaccinee	None	1379 ± 284	1299 ± 181		
2		PHA	37086 ± 2078	Not done	26.9	$P_{2-3} < 0.05$
3		PHA+F $(ab^1)_2$	23122 ± 4105	Not done	16.7	
4		TBE	Not done	2163 ± 173	1.7	$P_{4-5} < 0.05$
5		TBE+F $(ab^1)_2$	Not done	1340 ± 171	1.1	$P_{5-1} > 0.05$
6	JE vaccinee	None	360 ± 62	144 ± 16		
7		PHA	64447 ± 2469	Not done	137.8	
8		PHA+F $(ab^1)_2$	1029 ± 247	Not done	25.3	$P_{7-8} < 0.05$
9		JE	Not done	344 ± 42	2.3	$P_{9-10} < 0.05$
10		JE+$F(ab^1)_2$	Not done	160 ± 30	1.1	$P_{10-6} > 0.05$

Results of one of 9 experiments which yielded similar results. Lymphocytes were isolated 7 days after immunization.

suggesting that the blast transformation reaction could be used for evaluating the immunogenicity of antiviral vaccines.

Simultaneous addition to lymphocytes with viral antigen of 2 mg $F(ab^1)_2$ fragment of IgG completely suppressed the transformation up to the level found with non-stimulated lymphocytes (Table 2). In all instances this effect was related with the inhibitory effect of the $F(ab^1)_2$ fragment of phytohaemagglutinin-induced lymphocyte transformation. The existence of a common mechanism of suppression of the blast transformation by both phytomitogens and viral antigens can thus be assumed.

As to the mechanism of the phenomenon of inhibition by $F(ab^1)_2$ fragments of lymphocyte transformation, several explanations can be offered. According to one of them, $F(ab^1)_2$ fragments of normal human IgG interact with lymphokines. The consequence of such interaction could be a change in the functional activity of lymphokines (their effect on lymphocyte transformation). The presence of Fab-like structures in one of the lymphokines (lymphotoxin) was recently demonstrated by antibody to Fab fragments of immunoglobulin (Yamomoto *et al.*, 1979). According to another hypothesis, $F(ab^1)_2$ fragments interact with receptors on the surface of lymphocytes of certain subpopulation, this leading to activation of the suppressor functions. The possibility of binding Fab fragments by lymphocytes has been experimentally confirmed (Johnson *et al.*, 1975; Hofman *et al.*, 1977).

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