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ASSAY OF POTENCY OF INACTIVATED MEASLES VIRUS VACCINE BY THE ANTIBODY-COMBINING TEST[1]

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SUMMARY The antibody-combining test was developed as an *in vitro* test for measuring the antigenic potency of inactivated measles virus which stimulated neutralizing antibody production *in vivo*. The antibody-combining capacity varied in parallel with the antigen dilution, and the effective antigen dose was calculated by the Reed and Muench method as the dilution of antigen which reduced the neutralizing capacity of a given serum to 1 unit after absorption. This value was proportional to the dilution of the serum employed for the test and the antigenic potency *in vivo*, especially when 3 or 6 units of antibody were employed for the test.

INTRODUCTION

Measles virus inactivated by ultraviolet irradiation still possesses the ability to combine with neutralizing antibody (TOYOSHIMA *et al.*, 1960). On the other hand, an antibody-combining test has been applied in the determination of the antigenic potency of inactivated polio virus vaccines (KRECH, 1960).

For antigen-fractionation and vaccine-production, methods should be rapid, cheap and highly reproducible. Although the antigenic activity of non-infectious measles antigen can be determined by its ability to stimulate antibody production in guinea pigs (DEWITT and NOOK, 1960; HILLEMAN *et al.*, 1962; Warren *et al.*, 1962) and in mice (KUNITA *et al.*, 1965; TOYOSHIMA *et al.*, 1965), these tests are not very precise and take much time. Hence, attempts were made to develop a neutralizing antibody-combining test as an in vitro method for the determination of the antigenic potency of measles virus vaccine.

MATERIALS AND METHODS

1. *Virus*

The Toyoshima strain of measles virus grown in FL cell cultures was employed for neutralization tests.

2. *Vaccines*

Formalin-inactivated measles virus vaccines were employed for the test. The sources of the vaccines used are listed in Table 1.

3. *Immune serum*

Hyper-immune serum was obtained from a rabbit

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TABLE 1 *Sources of formalin inactivated vaccines employed for the antibody-combining test*

Lot	Virus strain	Tissue culture	Remarks	Mouse* ED50
Ref. II*	Toyoshima	FL		$4^{2.43}$
6S	Tanabe	Monkey kidney		ND
N. 1**	Tanabe	Monkey kidney		$4^{2.28}$
No. 2**	Tanabe	Monkey kidney	Dialized against	$4^{2.85}$
No. 5**	Sugiyama	Monkey kidney	PBS and then Hank's BSS	$4^{1.85}$

ND : Not determined
* Previously reported by TOYOSHIMA *et al.* (1965)
** Test lots of inactivated measles virus vaccine, Measles Vaccine Research Association, Japan.

TABLE 2 *Neutralizing antibody-combining test on lot 6S*

Vaccine \ Serum	Original dilution					
	800	1,600	3,200	6,400	12,800	25,600
2^{0}	+ + + +	+ + + +	+ + + +	+ + + +	ND	ND
2^{-1}	+ + + +	+ + + +	+ + + +	+ + + +		
2^{-2}	− − − −	+ + + +	+ + + +	+ + + +		
2^{-3}	− − − −	− − − +	+ + + +	+ + + +		
2^{-4}	− − − −	− − − −	+ + + +	+ + + +		
2^{-5}	− − − −	− − − −	− − − −	+ + + +		
2^{-6}	− − − −	− − − −	− − − −	− + + +		
Control (Hank's BSS)	− − − −	− − − −	− − − −	− − − −	+ + + +	+ + + +
Effective antigen dose	$2^{1.5}$	$2^{2.75}$	$2^{4.5}$	$\geqq 2^{6.33}$		

+ : CPE positive — : CPE negative
Effective antigen dose : Antigen dilution which reduces neutralizing antibody to 1 unit, calculated by the Reed-Muench method.

immunized by 2 intravenous inoculations of concentrated measles virus on days 0 and 63, and 3 intramuscular inoculations of virus emulsified in Freund's incomplete adjuvant on days 0, 21 and 42. The neutralizing antibody titer of this serum was 1 :19200 as measured by the standard neutralization test. The serum was heated at 56°C for 30 minutes, diluted to 1 :400 and stored at −20°C.

4. *Tissue culture*

Test tube cultures of FL cells were employed for virus titration and neutralization.

5. *Potency test in vivo*

Potencies of vaccines were determined by the " 3 week test " using mice (TOYOSHIMA *et al.*, 1965) and at least triplicate tests of each vaccine were made before determination of the mouse ED_{50}.

RESULTS

An example of the antibody combining test

Twofold serially diluted vaccines were mixed with twofold serially diluted serum in a checkerboard manner and the mixtures were incubated overnight at 4°C. The serum controls

contained Hanks' balanced salts solution (Hanks' BSS) in place of vaccine and were treated in the same manner. The mixtures were mixed with an equal volume of active virus containing approximately 100 TCD_{50} in 0.1 ml and incubated for a further 1 hour at room temperature. Each of the final mixtures was inoculated into 4 FL cultures using an inoculum of 0.2 ml and incubated at 37°C for observation. The effective antigen dose was calculated by Reed and Muench method as the vaccine dilution which reduced the neutralizing antibody titer of the added serum to 1 unit against 100 TCD_{50} of active virus.

The results of a preliminary experiment with lot 6S vaccine is illustrated in Table 2. The *in vivo* potecny of this vaccine had not been determined accurately, but it absorbed the neutralizing antibody effectively even with the lowest dilution of serum employed (12 units of neutralizing antibody in 0.1 ml of serum-vaccine mixture). A correlation was noted between the effective antigen dose and the dilution of serum employed.

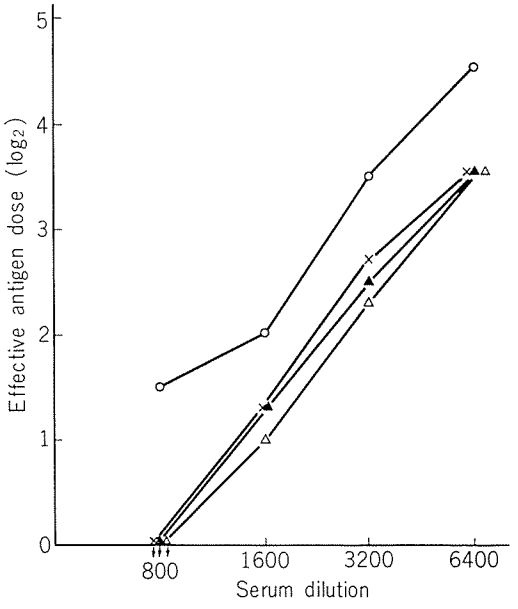

FIGURE 1 Antibody combining test of vaccines with different dilutions of serum.
▲——▲ No. 1 △——△ No. 5
○——○ No. 2 ×——× Ref. II

Comparison of the effective antigen doses of 4 vaccines and their antigenic potencies in vivo

Four different vaccines were tested their antibody-combining capacities by the same procedure. Before the test, lots No. 1, No. 2 and No. 5 vaccines were dialyzed at 4°C against phosphate buffered saline (PBS) for 2 days and then against Hanks' BSS for a day to remove merthiolate. With all these vaccines, roughly proportional correlations were again observed between the effective antigen dose and the dilution of the test serum (Fig. 1). Hence the effective antigen doses with each serum dilution were compared with the potencies of these vaccines determined by the mouse-potency test (Fig. 2). The antibody-combining capacities were well correlated with the ability to stimulate antibody production in mice, especially when 3 or 6 units of antibody was employed for the absorption test.

DISCUSSIONS

A rapid and precise method is required to determine the amount of antigen responsible for the production of neutralizing antibody during fractionation of antigens and production of vaccines. Although the hemagglutination test can be applied for this purposes, this test is not a direct measure of the amount of such antigen in addition to the disadvantage that the hemagglutinating activity is increased by disrupting the virus particles (NORRBY, 1963; FUNAHASHI and KITAWAKI, 1963; KITAWAKI *et al.*, 1964).

Attempts were made to apply a neutralizing antibody-combining test for the determination of antigenic potency. Since the antibody-combining capacity of inactivated virus is parallel to the virus dilution, the test was applied to 4 vaccines. Three of them, containing merthiolate as an antiseptic, were dialyzed before the test. The effective antigen doses

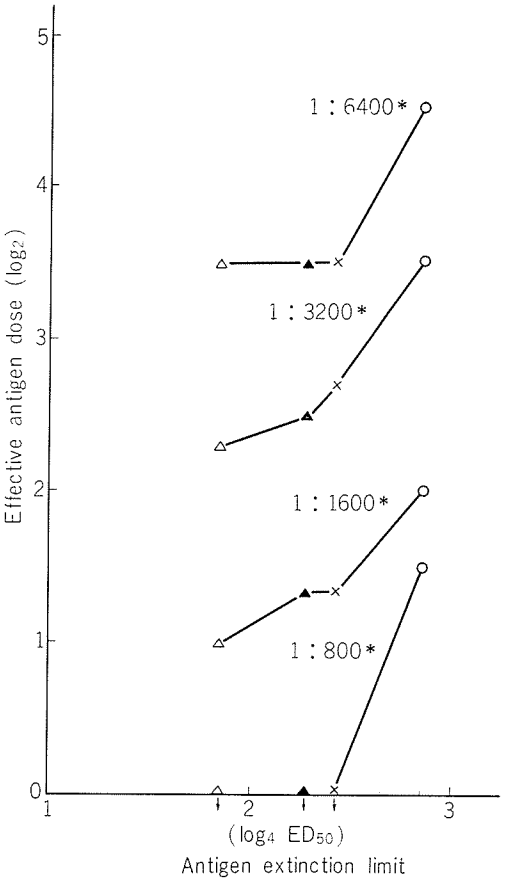

FIGURE 2 Comparison of effective antigen dose *in vitro* and antigen extinction limit *in vivo*.
▲ No. 1 △ No. 5
○ No. 2 × Ref. II
* Serum Dilution

determined by the antibody-combining test was roughly proportional to the serum dilution employed for the test. The effective antigen doses of these vaccines were compared with their abilities to stimulate antibody production in mice and were found to be well correlated with the latter, in spite of the change in volume of the vaccines during dialysis. When well-standardized virus and antiserum of a suitable dilution are applied in the antibody-combining test, the quantity of antigen may be determined more exactly by this test than by tests on animals which show differences in antibody response.

These results suggest the advantage of the antibody-combining test as a rapid method for evaluating the antigenic potentiality of inactivated measles virus. However, this test, like the animal test (TOYOSHIMA *et al.*, 1965), does not determine the efficacy of vaccine for practical use directly, but measures the antigen concentration.

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