APPLIED MICROBIOLOGY, Jan. 1968, p. 160-162

Vol. 16, No. 1
Printed in U.S.A.

Production of Specific Varicella Antiserum

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Received for publication 2 August 1967

Serological investigations of varicella have been limited because of difficulty in preparing specific immune serum. Varicella antiserum has been prepared in monkeys for fluorescent antibody purposes (N. J. Schmidt et al., J. Lab. Clin. Med. **66**:403, 1965). However, monkey serum often has antibody to other members of the herpesvirus group and frequently is anticomplementary. The recent report of the production of varicella-zoster complement-fixing antigen in AH-1 grivet monkey kidney cells (A. Svedmyr, Acta Pathol. Microbiol. Scand. **67**:159, 1966) stimulated a new attempt to produce experimental varicella antiserum.

The strain of varicella virus used was CP5,262. This strain was isolated at the National Communicable Disease Center (NCDC) by Philip Brunell from a typical case of childhood chickenpox. Virus inocula were in the form of infected cells sufficient to produce cytopathogenic effect (CPE) involving 70 to 90% of the culture in 5 to 7 days.

The stable AH-1 line of green monkey kidney (P. Gunalt, Proc. Soc. Exptl. Biol. Med. **118**:85, 1965) and diploid human fibroblasts of foreskin pool origin (FSP) were utilized. The tissue culture medium was a modified Eagle's medium (R. E. Kissling and D. R. Reese, J. Immunol. **91**:362, 1963), supplemented with 10% newborn calf serum for growth of cells or with 2% newborn calf serum for maintenance.

Unless otherwise specified, immunizing and complement-fixation (CF) antigens were produced from monolayer cultures in 32-oz (907-ml) prescription bottles. Fresh maintenance medium was placed on the cells the day after inoculation. When the desired degree of CPE was evident, the medium was replaced with 4 ml of Veronal-buffered saline (VB), *p*H 7.2, for CF purposes or with phosphate-buffered saline (PBS), *p*H 7.4, when immunizing antigens were desired. The cells were scraped into buffer solution and sonically treated in a glass bottle for 20 min in a 10-kc Raytheon sonic oscillator or directly in the cup of the instrument for 3 min. Uninfected cell cultures treated in the same way were used as control antigens.

A standardized micro CF technique was used (H. L. Casey, Part II, Adaptation of LBCF method to micro technique, *in Standardized Diagnostic Complement Fixation Method and Adaptation to Micro Test*. Public Health Monograph No. 74, Public Health Serv. Publ. 1228, 1965). CF antigens had optimal titers of 1:2 to 1:8 and were rarely anticomplementary.

Hartley strain guinea pigs of either sex, weighing 500 to 700 g, and New Zealand white rabbits weighing approximately 7 lb (3.2 kg) were used for the production of antisera.

Guinea pigs were routinely immunized with 0.2 ml of antigen given intraperitoneally on days 0, 2, 4, 7, 9, 11, and 18. When an adjuvant schedule was used, the guinea pigs received 1.0 ml intramuscularly of equal parts antigen and complete Freund's adjuvant plus a 0.2-ml booster without adjuvant on day 18. Sera were collected 7 to 10 days after the last inoculation.

Rabbits were inoculated with antigen consisting of virus produced in FSP cells and semipurified ("scrubbed") by sucrose density centrifugation. The first dose consisted of 2 ml of equal parts antigen and Freund's adjuvant given intramuscularly. An additional 1-ml dose of antigen without adjuvant was inoculated intramuscularly on day 27. Sera were collected 50 days after the last inoculation.

Sera from guinea pigs immunized with AH-1 and FSP antigens were tested by CF with antigens prepared from the heterologous cell line. Results are shown in Tables 1 and 2. The sera of animals receiving uninfected AH-1 antigen reacted at low titer to both infected and control antigens, whereas the sera of those receiving the infected AH-1 antigen reacted with undesirably high titers to the control antigen, although somewhat higher titers were observed against the varicella antigen. The FSP cell line proved to be more suitable for the production of immunizing antigens.

Purification of the serological antigen was attempted with discontinuous gradient centrifugation with the use of the following amounts and concentrations of sucrose: 5 ml, 80%; 3 ml, 65%; 5 ml, 50%; 10 ml, 35%; and 3 ml, 10%. On this was placed 5 ml of antigen. Centrifugation was carried out in an SW 25 rotor at 20,000

TABLE 1. *CF antibody titers of guinea pigs immunized with AH-1 antigens*

Guinea pig no.	Immunizing antigen	FSP CF antigen	
		Varicella	Control
1	Control AH-1 cells	<8[a]	<8
2		<8	<8
3		<8	<8
4		8	<8
5		8	8
6		8	8
7		8	8
8		8	8
9	Varicella-infected AH-1 cells	128	64
10		32	16
11		32	16
12		≥256	32
13		32	16
14		64	16
15		64	32
16		256	32
17		32	8

[a] Reciprocal of highest serum dilution producing 3 to 4+ fixation of complement.

TABLE 2. *CF antibody titers of guinea pigs immunized with FSP antigens*

Guinea pig no.	Immunizing antigen	AH-1 CF antigen	
		Varicella	Control
18	Control FSP cells	<8[a]	<8
19		<8	<8
20		<8	<8
21		<8	<8
22		<8	<8
23		<8	<8
24		<8	<8
25	Varicella-infected FSP cells	128	8
26		64	<8
27		256	8
28		128	<8
29		128	8
30		128	<8
31		128	8
32		64	<8

[a] Reciprocal of highest serum dilution producing 3 to 4+ fixation of complement.

rev/min for 3.5 hr. Samples were collected from the bottom of the tube and examined by electron microscopy. Many naked viral particles mixed with a few enveloped particles were observed at the 65 to 80% interface. Progressively more enveloped particles were observed at higher levels, until a maximum was observed in the sample collected at the 35 to 50% interface. Small numbers of both enveloped and naked virions were observed above the 35 to 50% interface. Subsequent centrifugal runs were made with gradients using 80, 60, 35, and 10% sucrose layers. Bands observed at the 60 to 35% sucrose interface were collected and used as CF or immunizing antigens after dialysis against the appropriate buffer. The optimal varicella antigen dilution was 1:2, with no activity in similarly prepared control antigen. Two guinea pig sera each from the group immunized with normal control and varicella-infected AH-1 cells were tested with the sucrose-centrifuged antigens. The control sera reacted to neither antigen, and the varicella antisera reacted only with the varicella antigen at titers of 1:64 and 1:128.

TABLE 3. *CF antibody titers of guinea pigs immunized with semipurified varicella antigen*

Guinea pig no.	Immunization schedule	AH-1 CF antigen	
		Varicella	Control
33	Adjuvant	≥256[a]	<8
34		≥256	<8
35		≥256	<8
36		≥256	<8
37		≥256	<8
38	Routine	32	<8
39		8	<8
40		64	<8
41		32	<8
42		32	<8

[a] Reciprocal of highest serum dilution producing 3 to 4+ fixation of complement.

Groups of five guinea pigs were immunized with semipurified antigen by use of both routine and adjuvant schedules. When sera of these animals were tested with antigens produced in AH-1 cells, suitable titers were observed with varicella and no reaction was observed with control preparations (Table 3).

Sera of those guinea pigs receiving sucrose "scrubbed" antigen by the routine schedule were also tested with standard NCDC herpesvirus hominis and vaccinia antigens and their appropriate controls. No reactions were obtained between these antigens and the varicella antisera.

The globulin fraction from immune rabbit sera was obtained by diethylaminoethyl cellulose

chromatography and conjugated with fluorescein isothiocyanate. The conjugate was passed through a Sephadex G-25 column and used without further treatment. Specific fluorescence was observed in varicella-infected AH-1 cultures stained with 1:2 and 1:4 dilutions and fluorescence with reduced brilliance was observed with 1:8 dilutions of the conjugates. Staining was most prominent in the cytoplasm of cells showing viral CPE, a finding compatible with the cytoplasmic location of enveloped virions as viewed by electron microscopy.