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Preparation of Type 2 Herpes Simplex Virus Complement-Fixing Antigen

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Comparable complement-fixing antigens of type 1 and type 2 herpes simplex virus were produced by extraction of infected African green monkey cells with 0.85% NaCl which was buffered at *p*H 9.0 with 0.05 M glycine-NaOH. The optimal antigen dilutions were higher in titrations against hyperimmune animal sera than in titrations against human sera. Complement-fixing antibody to type 2 herpes antigen was detected in 5 of 17 sera from healthy humans.

The complement fixation (CF) test has been used for a number of years as a routine test for the serological diagnosis of herpetic infection. In view of the more recent interest in the sero-epidemiological potential of evaluating humoral antibody responses in humans to type 1 and type 2 herpes simplex virus (HSV) (6), it has become pertinent to define the value and limitations of this test in differentiating herpes type-specific antibody in human sera.

The unavailability of an adequate CF antigen has limited serological studies of type 2 herpes by CF in our laboratory. Crude tissue culture antigens of type 2 herpes prepared by the same method as type 1 herpes antigens have generally been of low CF titer, and their specificity has been dubious. The recent finding of Krech and Jung (5) which demonstrated that similar difficulties in preparing cytomegalovirus CF antigen could be overcome by glycine extraction of infected cells at high *p*H stimulated new attempts to prepare potent type 2 herpes CF antigen for use in comparative serological studies.

CF antigens were prepared from monolayer cultures of continuous African green monkey cells (Vero) infected with the VR no. 3 strain of type 1 or MS strain of type 2 HSV (9, 3). The tissue culture medium was a modified Eagle's (4) medium supplemented with 10% fetal calf serum for growth of cells or with 2% serum for maintenance and propagation of virus. Viruses were inoculated at a multiplicity sufficient to cause 100% infection in 48 hr at 35 C. At 48 hr a cytopathic effect was evidenced by rounded, refractile cells in coalescing foci and many infected and lysed cells in the culture fluid. The cell sheet remaining on the glass was scraped into the culture medium, and the suspension was centrifuged at 28,000 rev/min for 1 hr in a no. 30 Spinco rotor. The pellet from this centrifugation was resuspended in sufficient 0.85% NaCl, buffered at *p*H 9.0 with 0.05 M glycine-NaOH, to effect a 20-fold concentration of the original material; this suspension was then treated sonically for 10 min in a Ratyheon sonic oscillator. The sonically treated material was used as CF antigen without further treatment. Uninfected cell cultures treated the same way were used as control antigens. CF tests were performed with these antigens by the LBCF method (1). This method defines the optimal antigen dilution as that which gives the greatest amount of complement fixation with specific antiserum without being anticomplementary.

Table 1 presents data derived from titrations of VR no. 3 and MS CF antigens for determination of optimal antigen dilutions. The sera included in this table are hyperimmune rabbit anti-type 1 and anti-type 2 HSV sera and human sera typed as HSV type 1 or type 2 by the micro indirect hemagglutination (2) and quantal neutralization (8) methods. The data in Table 1 show that the optimal dilution of both herpes types was 1:32 when the two antigens were titrated against hyperimmune rabbit anti-HSV serum. However, the optimal dilution was 1:16 when the VR no. 3 antigen was titrated against a human anti-type 1 serum, and the MS antigen was titrated only 1:4 against its homologous human antiserum. It is therefore suggested that the appropriate antigen dilutions for HSV type 1 and type 2 CF antigens be determined for the particular system in which they are to be used. We have consistently noted that the optimal dilution of antigen will be higher when it is tested against a hyperimmune animal serum than against a human serum.

Table 2 shows the results of CF tests with op-

TABLE 1. *Optimal VR no. 3 and MS complement-fixing antigen dilutions as determined by the LBCF (1) method*

Antigen	Dilution	Rabbit anti-type 1 HSV[a]					Rabbit anti-type 2 HSV					Human anti-type 1 HSV					Human anti-type 2 HSV				
		16	32	64	128	256	512	16	32	64	128	256	512	16	32	64	128	256	512	16	32	64	128	256	512
VR #3	4	4	4	4	4	0	0	4	4	4	4	0	0	4	4	4	2	0	0	4	4	±	0	0	0
	8	4	4	4	4	0	0	4	4	4	4	±	0	4	4	4	4	0	0	3	±	0	0	0	0
	16	4	4	4	4	3	0	4	4	4	4	1	0	4	4	4	4	±	0	3	±	0	0	0	0
	32	4	4	4	4	4	±	4	4	4	3	±	0	4	4	4	1	±	0	1	0	0	0	0	0
	64	4	4	3	3	2	0	4	4	4	2	0	0	4	4	3	±	0	0	±	0	0	0	0	0
MS	4	4	4	2	0	0	0	4	4	4	4	2	0	4	4	2	0	0	0	4	4	4	±	0	0
	8	4	4	1	0	0	0	4	4	4	4	3	0	4	1	±	0	0	0	4	4	2	0	0	0
	16	4	4	1	0	0	0	4	4	4	4	4	0	4	1	0	0	0	0	4	4	3	0	0	0
	32	4	2	±	0	0	0	4	4	4	4	4	0	4	1	0	0	0	0	4	2	±	0	0	0
	64	4	±	0	0	0	0	4	4	4	4	0	0	4	1	0	0	0	0	4	2	0	0	0	0

[a] Symbols: 4, 0% hemolysis; 3, 30% hemolysis; 2, 50% hemolysis; 1, 75% hemolysis; ±, 90% hemolysis; 0, 100% hemolysis.

TABLE 2. *Complement-fixing antibody titer[a] to VR no. 3 and MS herpes simplex virus in sera of healthy humans*

Serum no.	HSV VR no. 3 titer	HSV MS titer
1	8	—[b]
2	32	—
3	—	—
4	16	—
5	32	8
6	16	—
7	8	—
8	32	—
9	—	—
10	8	—
11	32	16
12	32	16
13	16	—
14	32	—
15	32	—
16	64	32
17	64	8

[a] Reciprocal of serum dilution.
[b] Serum CF titer of less than 1:8.

timal dilutions of VR no. 3 and MS glycine-extracted antigens tested against 17 sera from healthy adults over the age of 40 years. These data show that 15 of the 17 sera contain CF antibody levels $\geq$ 1:8 to type 1 herpes and 5 of the sera contain similar levels of antibody to type 2 herpes. Sera 5, 11, 12, 16, and 17 contain CF antibody levels $\geq$ 1:8 against both HSV types. Two of the sera (3 and 9) have no detectable antibody to either herpes type, and none contain antibody only to type 2 herpes.

It is pertinent to add that other laboratories have differentiated HSV type-specific antibody in humans with primary infection by CF but found the test less sensitive than the quantal microneutralization method for detecting prior exposure to both HSV types (7). However, we believe that the CF test can be a useful sero-epidemiological method for determining prior infection with these viruses if adequate antigens are used in the tests. The method described in this study for preparation of potent type 2 herpes CF antigen and its proper titration before use should facilitate evaluation of the CF test in differentiating HSV type-specific antibody in human sera. To this end, studies comparing the quantal microneutralization, micro indirect hemagglutination, and CF tests in detecting and differentiating HSV antibody are in progress.

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