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Improved Glycine-Extracted Complement-Fixing Antigen for Human Cytomegalovirus

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High-titered, sensitive, and stable complement-fixing antigens for human cytomegalovirus were consistently produced from human diploid fibroblast cells infected at a high multiplicity of virus, harvested after 7 days of incubation, and sonically treated immediately in 0.1 M glycine buffer, pH 9.5.

Complement-fixing (CF) antigens for human cytomegalovirus (CMV) produced by glycine buffer extraction of infected cells (GE antigens) have proved to be more sensitive for antibody detection than are antigens prepared by freezing and thawing infected cells (1, 4). However, the optimal dilutions of GE antigen for antibody assay, as determined by box titrations, are generally in the range of 1:2 or 1:3, making them relatively expensive to use for routine serodiagnosis. Further, GE antigens may exhibit anticomplementary (AC) or nonspecific activity at these low dilutions. Therefore, efforts were made to produce more potent GE antigens for CMV.

The AD-169 strain of human CMV was propagated in lines of human fetal diploid lung (HFDL) cells developed in this laboratory by standard procedures (2), and high-titered cell-free CMV preparations (6) were used to infect cultures for antigen production. CF tests were performed by our standard procedure (5), and CMV antigen from Flow Laboratories (lot W-946069) was used as a reference preparation.

The importance of using a large infecting dose of virus for antigen production was demonstrated by experiments shown in Table 1. Cultures infected at a multiplicity of infection (MOI) of 1 plaque-forming unit per cell and harvested at 6 and 7 days produced the greatest amount of antigen. These heavily infected cultures showed an early, rounded cytopathic effect (CPE) at 24 h. High-titered cell-free seed virus, rather than cell-associated virus, was essential for initiating a massive and synchronous infection of the HFDL cells. Antigens produced in cultures infected at a low MOI showed some AC activity.

Comparative studies showed that CMV-infected cells lost appreciable amounts of antigen into the suspending medium when they were stored frozen prior to extraction with alkaline buffer. Therefore, CMV GE antigens were prepared from freshly harvested infected cells.

Extraction of CMV-infected cells with glycine buffer alone, as well as by combinations of sonic and glycine buffer treatments, was compared for efficacy in releasing antigen, and the results of representative experiments are shown in Table 2. Based upon antigen titers achieved, ease of preparation, and freedom from AC activity, infected cells were suspended in glycine buffer and sonically treated immediately.

The following procedure was adopted for routine preparation of CMV GE antigen. HFDL monolayer cultures in 32-ounce (ca. 0.946 liter) bottles were infected at an MOI ≥ 1 in Eagle minimal essential medium with 10% fetal bovine serum; after 3 to 4 days at 36°C the medium was changed, and incubation was continued for a total of 7 days. Cells were dislodged into the medium by shaking with glass beads, sedimented by centrifugation at 1,500 rpm for 15 min, suspended in 1/20th of the original culture volume of 0.1 M glycine buffer, pH 9.5, and disrupted by sonic treatment. A Biosonik II apparatus was used with the probe intensity set at 50%. The cell suspension (in a plastic tube in a wet-ice bath) was treated with three 10-s bursts followed by a 5-s cooling period for a total of 30 s of sonic treatment. After centrifugation at 2,000 rpm for 20 min at 4°C, the supernatant fluid (GE antigen) was stored in convenient working volumes at −70°C. Control antigen was prepared in the same manner from uninfected-cell cultures of the same lot.

Electron microscopy showed that antigen prepared in the above manner resembled other GE antigens (1) in containing predominantly nucleocapsids. It contained enveloped virions at about a 10-fold lower concentration and small dense bodies showing breakdown of their membranes. In contrast, a freeze-thaw preparation examined

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TABLE 1. *Effect of MOI on CF titers of alkaline buffer extracts[a] of CMV-infected HFDL cells*

Day of harvest	MOI used to infect cell cultures					
	1.0		0.1		0.01	
	CPE	Antigen titer[b]	CPE	Antigen titer	CPE	Antigen titer
2	4+	1:2	2+	<1:2	±-1+	<1:2 AC[c]
3	4+	1:2	2+	1:2	±-1+	1:2 AC
4	4+	1:4	3+	1:2	1+	1:2 AC
5	4+	1:4	3+	1:2 AC	1+	1:2 AC
6	4+	1:8	4+	1:2	1+	1:2 AC
7	4+	1:8	4+	1:4	2+	1:2 AC

[a] Infected cells were stored at −70°C in physiological saline for 7 days, then washed and incubated in 1/20th of the original culture volume of 0.1 M glycine buffer, pH 9.5, for 6 h at 37°C. The fluid phase (extract) was assayed for CF activity.
[b] One unit of antigen as determined in a box titration.
[c] Antigen showed AC activity at dilution containing 1 antigenic unit.

TABLE 2. *Comparison of CF antigen titers obtained by extracting CMV-infected cells by various procedures*

Treatment	CF antigen titers[a]	
	Expt 1	Expt 2
Incubation in glycine buffer 37°C 6 h	1:4	1:4
Sonic treatment in glycine buffer frozen immediately	1:32	1:16
Sonic treatment in glycine buffer followed by 37°C 6 h	1:16	1:16
Incubation in glycine buffer 37°C 6 h followed by sonic treatment	1:16	1:8

[a] One unit of antigen as determined in a box titration.

in parallel contained roughly equal numbers of enveloped virions and intact dense bodies, and nucleocapsids at about a 10-fold lower concentration.

GE antigens produced by the new procedure were as sensitive as the reference antigen in demonstrating diagnostically significant increases in CMV antibody titer (Table 3) and in detecting antibody in individual sera (Fig. 1).

High-titered CMV antigens were produced consistently by the new method. Of five antigen lots prepared in the L-645 cell line, three had titers of 1:16, two had titers of 1:32, and two lots prepared in the L-7503 cell line had titers of 1:16. These antigens could be used at dilutions of ≥1:8, as compared to 1:2 or 1:3 for previous preparations.

Stability studies on antigen samples stored in parallel at 4, −20, and −70°C and titrated at various time intervals showed that antigen titers and sensitivity for detecting antibody were stable at all three temperatures for at least 60 days, the longest period tested. Thus, convenient working volumes of antigen can be thawed and held at 4°C, but for long-term storage −70°C is probably preferable.

CMV antigens prepared as described are more reliable and economical reagents for serodiagnosis than those previously available. Activity of these antigens cannot be accurately compared with that of antigens recently described by other workers (3), since their antigen end points were based upon titrations against a constant 4 U of

TABLE 3. *Ability of sonically treated GE antigen to detect diagnostically significant increases in CMV CF antibody titer*

Patient	Age (Sex)	Clinical syndrome	Days after onset	CF antibody titer vs.	
				Sonically treated GE antigen (1:8)	Reference antigen (1:3)
CaEb	24 (F)	Recurrent fever	9	<16	<16
			19	1,024	256
CyCo	31 (F)	None		<16	<16
				256	64
DoMe	31 (M)	Fever following transfusions	4	<16	<16
			25	512	512
KaCu	44 (M)	Pneumonitis	20	<16	<16
			31	32	16
AlSt	52 (M)	Fever, kidney rejection	17	<16	<16
			46	64	32

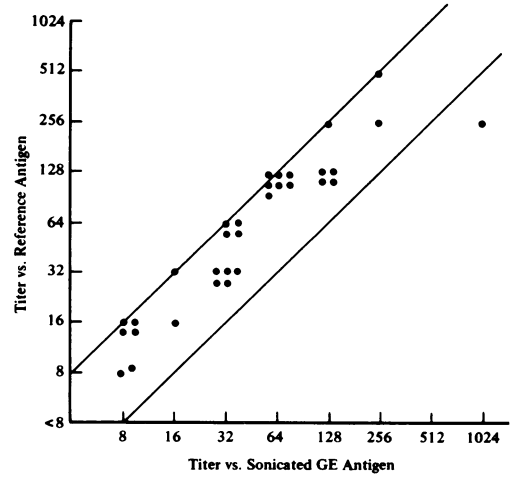

FIG. 1. *Comparison of antibody titers obtained with sonically treated GE antigen and with reference antigen in routine CF runs. Diagonal lines enclose titers differing by no more than ±1 twofold dilution.*

antiserum, whereas our antigens were evaluated on the basis of optimal reactivity in box titrations.

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