Complement-Fixing Antigens Produced by Varicella-Zoster Virus in Tissue Culture

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The complement-fixing antigens present in tissue cultures infected with varicella-zoster virus were separated into four components. There were (i) a cell-free soluble antigen, (ii) a cell-associated soluble antigen, (iii) a cell membrane-associated antigen, and (iv) a virion antigen. All four antigens were reactive with sera from patients with varicella or zoster, and about 90% of the total complement-fixing activity was found to be nonvirion-associated.

Complement-fixing (CF) antigens for use in the serological diagnosis of varicella-zoster virus (VZV) infection are usually prepared from the cellular phase of infected cultures and presumably contain all of the antigens produced by the virus (6, 7). Although the antigens are satisfactory for use as diagnostic reagents, little information is available on the nature of the antigenic components present in the crude antigen preparations. Early workers reported the presence of a soluble VZV antigen in vesicular fluids (3), but later workers found no definite evidence for the presence of an antigen which was separable from the virus in infected cell culture fluids (6). To more adequately characterize the antigenic nature of VZV, we separated a total of four major antigenic components from VZV-infected cell cultures and tested these antigens in CF tests against sera from patients with varicella or zoster. These antigens consisted of two soluble and two particulate antigens, and about 90% of the total CF activity in VZV-infected cell cultures was found to be nonvirion-associated.

(i) Crude cell-associated antigen. The cell sheets from the above described VZV-infected cell cultures were rinsed with phosphate-buffered saline, and then were scraped into glycine-saline buffer, pH 9.0, with a rubber scraper. The cells were then sonically treated for 20 min in a Raytheon 10-kc sonic oscillator. This sonicate constituted crude VZV cell-phase CF antigen.

(ii) Cell-associated soluble antigen. The crude VZV cell-phase CF antigen was then layered onto a 3-ml cushion of 30% (wt/vol) sucrose and was centrifuged for 2 h at 30,000 rpm in an SW40 Spinco rotor. The supernatant fluid above the sucrose cushion was collected and used as cell-associated soluble antigen.

(iv) Viral and cell membrane-associated antigens. The pellet from the centrifugation was resuspended in Veronal buffer and layered onto linear 30% to 50% glycerol-potassium tartrate viscosity-density gradients (J. F. Obijeski et al., in press) prepared in Veronal buffer and centrifuged for 18 h at 39,000 rpm in an SW40 Spinco rotor. Equilibrium was reached within 3 h when centrifugation was carried out at this speed. This centrifugation procedure was similar in principle to the positive density-negative viscosity gradients described by Barzilai, Lazarus, and Goldblum (1).

CF activity was associated with the two visible bands in the gradients. Each band was monitored by electron microscopy to determine presence or absence of virions or nucleocapsids. The upper, hazy band, which contained no virus particles, appeared as homogeneous, membrane-like material. It was designated cell membrane antigen. The lower, stringy band was composed predominantly of complete, enveloped particles and a few nucleocapsids, and was designated viral antigen. These bands were collected by puncturing the side of the tube with a needle and dialyzed against Veronal buffer.

Concurrent with the preparation of VZV antigens, noninfected cells were treated in the same way as infected cultures and were used as antigen controls.
**Sera and CF test.** Sera from patients in the acute or convalescent stage of VZV infection were obtained from the Vesicular Disease Section, Center for Disease Control. Hyperimmune guinea pig sera to VZV prepared against virus propagated in monkey kidney cells were obtained from Robert Kissling (4). CF tests were performed by the microtiter laboratory branch complement fixation (LBCF) method (2).

**Electron microscopy.** Specimens were prepared for electron microscopy by the pseudoreplica technique. They were stained with 0.5% uranyl acetate and examined with a Philips 200 electron microscope.

**RESULTS**

In CF tests with different antigenic fractions present in VZV-infected cell cultures, each antigen was tested by block titration by the LBCF microtiter method against human convalescent-phase serum obtained from patients with clinically diagnosed VZV (Table 1). As shown, the crude cell antigen had a total of 9,600 CF units. Sixty percent of this total activity was recovered in the cell-associated soluble-antigen fraction; 35% was recovered in the cell membrane fraction; and the rest, 11%, was recovered in the virus antigen. More than 100% of the total activity was recovered by the procedures used in the fractionation procedure. The culture fluid also contained more total CF units than were recovered in the crude cell antigen (Table 1). Control antigens were not reactive with any of the sera tested in this study.

In a glycerol-potassium tartrate viscosity density gradient of VZV particulate antigen, two bands were evident (Fig. 1). The upper diffuse band is composed of cell membrane material, whereas the lower band contains virus particles. An electron micrograph of the virus band is shown in Fig. 2. The particles are predominantly virions, with some particles sufficiently penetrated by stain to reveal nucleocapsids. Electron microscopic examination of all other antigen fractions showed that they did not contain virions or nucleocapsids.

CF tests were performed with five different VZV CF antigens against VZV acute- and convalescent-phase sera from patients with zoster and against hyperimmune, VZV guinea pig sera (Table 2). These data indicate that each antigen is highly reactive with VZV antibody. Fourfold diagnostic antibody rises were detected with all antigens tested against four different paired sera. However, the cell-associated soluble antigen appeared slightly less reactive than the rest with both human and guinea pig anti-VZV sera.

Antibody to the soluble antigen was present in sera of patients with varicella or zoster within

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**TABLE 1. Complement-fixing (CF) activity of the antigenic components present in varicella-zoster virus-infected human diploid fibroblast cell cultures**

| Antigen                        | Vol (ml) | Optimal antigen dilution | CPU/ml | Total CFU | Total CFU (%) |
|-------------------------------|----------|--------------------------|--------|-----------|---------------|
| Crude cell antigen            | 30       | 1:8                      | 320    | 9,600     | 100           |
| Cell-associated soluble antigen | 36       | 1:4                      | 160    | 5,760     | 60            |
| Cell membrane antigen         | 22       | 1:4                      | 160    | 3,520     | 35            |
| Partially purified virus antigen | 7        | 1:4                      | 160    | 1,120     | 11            |
| Total CFU recovered (%)       |          |                          |        |           |               |
| Cell-free soluble antigen, 20× concn* | 75       | 1:4                      | 160    | 12,000    | 106           |

* CF activity not detectable in unconcentrated culture fluids.

**Fig. 1. Photograph of 30 to 50% glycerol-potassium tartrate gradient after centrifugation of VZV particulate antigen. Arrow indicates virus band.**

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2 weeks after the onset of rash and remained relatively high for about 3 months (Table 3).

We also noted that the VZV cell-free soluble antigen was not reactive with some sera which exhibited fourfold antibody rises to both VZV and herpes simplex crude virus (HSV) antigen. Serum pairs 1, 2, and 3 exhibited fourfold antibody rises to both VZV and HSV antigen, but not to VZV cell-free soluble antigen (Table 4). The convalescent sera of serum pairs 1 and 3 both showed a titer of 8 when tested with HSV cell-free soluble antigen. These sera were from patients with clinical disease compatible with HSV infection. Serum pairs 4 and 5 were from patients with clinically diagnosed zoster. They also exhibited fourfold antibody rise to both viral antigens and, in addition, had a fourfold antibody rise to the VZV soluble antigen.

DISCUSSION

Previous investigators have shown that a soluble VZV CF antigen is present in zoster vesicular fluids (3), but others could not confirm this finding with culture fluids from VZV-infected cells (6). The data presented in this study are at variance with those of the latter

Table 2. Reactivity of varicella-zoster virus antigenic components in complement-fixing (CF) tests against sera from patients with clinically diagnosed herpes zoster infection and against hyperimmune VZV guinea pig antiserum

| Sera | CF titer with indicated antigen |
|------|--------------------------------|
|      | Crude cell extract | Cell-associated soluble antigen | Cell-membrane-associated antigen | Partially purified virions | Cell-free soluble antigen |
| (1) S₀ | 8 | — | — | — | 8 |
| S₂ | 128 | 64 | 128 | 128 | 128 |
| (2) S₀ | 32 | 64 | 32 | 32 | 8 |
| S₂ | 512 | 256 | 512 | 512 | 512 |
| (3) S₀ | 8 | — | — | — | — |
| S₂ | 512 | 512 | 512 | 512 | 512 |
| (4) S₀ | 8 | — | — | — | — |
| S₂ | 512 | 128 | 256 | 256 | 256 |
| (5) S₂ | 512 | 512 | 512 | 512 | 512 |
| Guinea pig | 64 | 32 | 64 | 64 | 64 |

*a End point dilution factor.
*b Denotes a titer of less than 8.

Table 3. Complement-fixation (CF) antibody titers* of sera from patients with varicella or zoster to varicella-zoster (VZ) viral and cell-free soluble antigen

| Clinical diagnosis | Serum | Days after onset of rash | CF titer with indicated antigen |
|--------------------|-------|--------------------------|--------------------------------|
|                    |       |                          | VZ viral | VZ cell-free soluble |
| Varicella          | S₀    | 0 | —b | — |
|                    | S₂    | 14 | 64 | 32 |
| Varicella          | S₀    | 20 | 128 | 64 |
|                    | S₂    | 25 | 128 | 64 |
| Varicella          | S₀    | 26 | 128 | 32 |
|                    | S₂    | 32 | 128 | 64 |
| Varicella          | S₀    | 13 | 128 | 128 |
|                    | S₂    | 52 | 128 | 64 |
|                    | S₂    | 92 | 64 | 16 |
| Zoster             | S₀    | 7 | — | — |
|                    | S₂    | 41 | 256 | 128 |
| Zoster             | S₀    | 15 | 64 | 128 |

*a End point dilution factor.
*b Denotes a titer of less than 8.
Table 4. Homologous and heterologous complement-fixing (CF) titer* of serum from patients with herpetic infection tested with herpes simplex (HS) and varicella-zoster (VZ) viral and cell-free soluble antigens

| Virus (Clinical diagnosis) | Patient and serum | CF titer with indicated antigen | HS viral | HS cell-free soluble | VZ viral | VZ cell-free soluble |
|----------------------------|-------------------|---------------------------------|----------|---------------------|----------|---------------------|
| Herpes simplex             | (1) $S_1$, $S_2$ | 16                              | 8        | 256                 | 64       | 256                 |
| Herpes simplex             | (2) $S_1$, $S_2$ | 8                               | 32       | 8                   | 64       | 256                 |
| Herpes simplex (isolate)   | (3) $S_1$, $S_2$ | 256                             | 8        | 256                 | 16       | 16                  |
| Herpes zoster              | (4) $S_1$, $S_2$ | 32                              | 64       | 16                  | 64       | 64                  |
| Herpes zoster              | (5) $S_1$, $S_2$ | 32                              | 64       | 16                  | 64       | 64                  |

* End point dilution factor.
* Denotes a titer of less than 8.

workers, because a soluble antigen was readily demonstrable in the culture fluids of VZV-infected cells which had been freed of virus by ultracentrifugation. However, to demonstrate this activity we found it necessary to use serum-free culture fluid and to concentrate the fluids 20-fold. Furthermore, we observed that over one-half of the total CF activity present in crude VZV cell antigen was associated with a cell-associated soluble antigen. Another 35% was associated with cell membrane material, and the rest, with virus particles. The cell-associated soluble antigen is probably composed of virion precursor material, and the cell membrane material undoubtedly contains virion envelope proteins similar to those found in membranes derived from HSV-infected cells (5).

When optimal dilutions of each separated VZV antigen are used in CF tests against VZV antisera, the antigens are found to be about equally reactive in detecting antibody, although the cell-associated soluble antigen may be slightly less reactive than the rest. One of the antigens, the cell-free soluble antigen, may prove to be of value in serologically differentiating VZV from HSV when sera contain diagnostic antibody levels to both viruses. Preliminary studies indicate that diagnostic antibody rises to the VZV soluble antigen may be indicative of recent or current VZV infection, and in limited tests with sera from patients with antibody rises to both HSV and VZV, the VZV cell-free soluble antigen has been found to react only with sera from patients with VZV infection. Although optimal antigen dilutions were used in the diagnostic tests, block titrations of the antigens against human and animal anti-VZV and anti-HSV sera showed no cross-reactivity at any dilution. Studies are in progress to ascertain the value of using VZV component antigens in the differential serological diagnosis of herpetic infection and in elucidating the primary factor responsible for the VZV-HSV heterotype antibody responses which now complicate and limit the value of the CF test in such diagnoses (7).

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