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Varicella-Zoster Virus Fails to Induce Immunoglobulin G Fc Receptors in Infected Human Cells (41265)

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Abstract. Varicella-zoster virus infection of human fibroblasts and human brain cells produces characteristic cytopathogenic effects and virus-specific antigens. Receptors for the Fc portion of immunoglobulin G, which have been noted on cells infected with other human herpesviruses, could not be detected either by hemadsorption of sensitized sheep erythrocytes or by binding of radiolabeled IgG. Herpes simplex virus-infected cells were positive under the same conditions.

Herpes simplex virus (HSV) and human cytomegalovirus (CMV) both induce receptors for the Fc portion of IgG on the surface of infected cells (1, 2). These receptors appear to be coded by the virus, rather than the host cell (3). They are postulated to be important in the development of latent infection (4, 5) and they are able to protect the infected cell from complement-mediated and cell-mediated lysis (6). Binding of virus-specific antibody by both the Fab and Fc portions has also been shown to suppress viral replication within infected cells (7).

Varicella-zoster virus (VZV) is another human herpesvirus which exhibits latency in man (8). Cells infected with VZV have not been adequately examined for Fc receptors. Such information is important not only for its implications as to the mechanism of VZV latency but also for the interpretation of immunofluorescence and other immunologic tests for virus-specific antigens. This report describes our inability to detect Fc receptors in VZV-infected cultured human cells.

Materials and Methods. *Virus.* Varicella-zoster virus (Ellen strain) was obtained from the American Type Culture Collection and propagated in Flow 5000 human embryonic fibroblasts (Flow Laboratories, Rockville, Md.). The virus was passaged as infected cells after release from the support surface with a mixture of 0.25% trypsin and 0.1% versene when 3–4+ cytopathic effect (CPE) was observed. The infected cells were added to confluent monolayers of Flow 5000 cells at a ratio of approximately 1:3. A second VZV strain was isolated in our laboratory from the vesicles of a patient with chickenpox and passaged in a similar fashion. Herpes simplex virus was also isolated in our laboratory from the vesicles of a patient with herpes labialis and propagated in Flow 5000 cells. Virus inoculum was prepared from the cell medium when CPE was maximal. Flow 5000 human embryonic fibroblasts were obtained from Flow Laboratories as noted above.

Cells. Human brain cells (M-787-CG and M-622-CG) were obtained from Bengt Westermark via Luis Glaser, Washington University School of Medicine, St. Louis, Missouri. These cells were derived from brain biopsies and prepared by the method of Pontén and MacIntyre (9). Their exact nature is unknown as they contain both glial markers (S-100) and the typical sodium channels of neuronal cells (10). All cells were carried in Eagle's minimum essential medium supplemented with 10% fetal calf serum (KC Biological, Lenexa, Kans.), 100 units/ml penicillin, 0.25 μg/ml Fungizone, and 100 μg/ml streptomycin. They were split 1:2 at confluence. Flow 5000 cells were carried and passaged in an identical fashion.

Binding of sensitized sheep erythrocytes. Human brain cells and Flow 5000 fibroblasts were grown to confluence on plastic coverslips in Leighton tubes (Costar, Cambridge, Mass.). At confluence, they were

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infected with either infected human fibroblasts (VZV) or an aliquot of the infected culture medium (HSV). Control cells were mock-infected. At 3+ CPE (75-90% of cells infected), the medium was discarded and the cells were washed with Hanks' balanced salt solution (BSS). The cells were incubated with a 1:50 dilution of rabbit anti-sheep red blood cell IgG (Cappel Laboratories, Cochranville, Pa.) in BSS for 1 hr at 37°. The cells were again washed with BSS and reincubated with a 0.5% suspension of sheep red blood cells (Flow Laboratories) in BSS for 30 min at 37°. Following this, the cells were washed three times in BSS and scored for hemadsorption under low power magnification. Permanent slides were fixed with 1.25% glutaraldehyde and stained with Giemsa.

Binding of ^{125}I-IgG. Human and rabbit IgG and $F(ab')_2$ fragments were purchased from Cappel Laboratories. They were labeled with ^{125}I (New England Nuclear, Boston, Mass.) using the chloramine-T procedure (11). All labeled materials were extensively dialyzed against phosphate-buffered saline (PBS). Greater than 99% of the radioactivity was protein bound. Specific activities were: human IgG, 6.35×10^5 cpm/μg (153.2 μg/ml); human $F(ab')_2$, 8.85 $\times 10^4$ cpm/μg (222.9 μg/ml); rabbit IgG, 8.83×10^4 cpm/μg (270.7 μg/ml); and rabbit $F(ab')_2$, 1.03×10^6 cpm/μg (143.6 μg/ml). Each preparation was heat aggregated at 63° for 15 min and 0.075- to 0.15-ml aliquots were added to duplicate VZV-infected, HSV-infected, or uninfected Flow 5000 cells in a 75-cm^2 culture flask containing 3 ml of fresh medium. The infected cells exhibited 3+ CPE (75–90% involvement) at the time of testing. The flasks were incubated at 37° for 2 hr and washed five times with fresh medium, and then the medium was removed. The cells were removed from the flask with 5 ml of 0.25% trypsin–0.1% versene, pelleted at 1000*g* for 10 min, and taken up in 0.5 ml of fresh medium. The tubes were then counted in a Packard gamma scintillation counter.

Immunofluorescence. Indirect immunofluorescence for the detection of VZV-specific antigens was done with standard techniques (12). A dilution of human convalescent zoster serum in PBS which produced 3+ fluorescence within the visible plaques of VZV-infected human fibroblasts was used for analysis. Varicella-zoster-infected human brain cells grown on glass coverslips were washed in PBS, acetone-fixed, and incubated with the zoster serum (30 min at 20°). The coverslips were washed in PBS three times and reincubated with fluoroscein isothiocyanate-conjugated goat anti-human IgG (Cappel Laboratories). The coverslips were again washed in PBS, air dried, and mounted with buffered glycerol. VZV-infected human fibroblasts, uninfected human brain cells, and negative serum were included as positive and negative controls in each test.

Results. Varicella-zoster virus-infected human fibroblasts do not develop Fc receptors as shown in Fig. 1A. Similar results were found with both virus strains. Likewise, no Fc receptors could be detected when other diploid human fibroblasts (IMR-90 and WI-38, ATCC) were used for infection or when the time of analysis was varied from just after inoculation to the point of complete cell sheet destruction. Herpes simplex-infected cells were analyzed for Fc receptors as a positive control. As shown in Fig. 1B, those cells with CPE were all positive. The single-step procedure, using presensitized sheep erythrocytes, also gave identical results but was thought to be harder to interpret because of red blood cell clumping.

Varicella-zoster virus infection of fibroblasts does not duplicate *in vivo* infection very well, however. Natural infection is characterized by replication in epithelial cells and abundant, stable, cell-free virus (13). Replication in fibroblasts, on the other hand, results in only small amounts of labile, strongly cell-associated virus (14). We therefore looked for VZV induction of Fc receptors in human brain cells, since neural tissue is the presumed site of viral latency and the virus will replicate in these cells (15). Varicella-zoster infection of both M-787-CG and M-622-CG cells did produce characteristic viral CPE. There was no evidence of Fc receptor induction, however

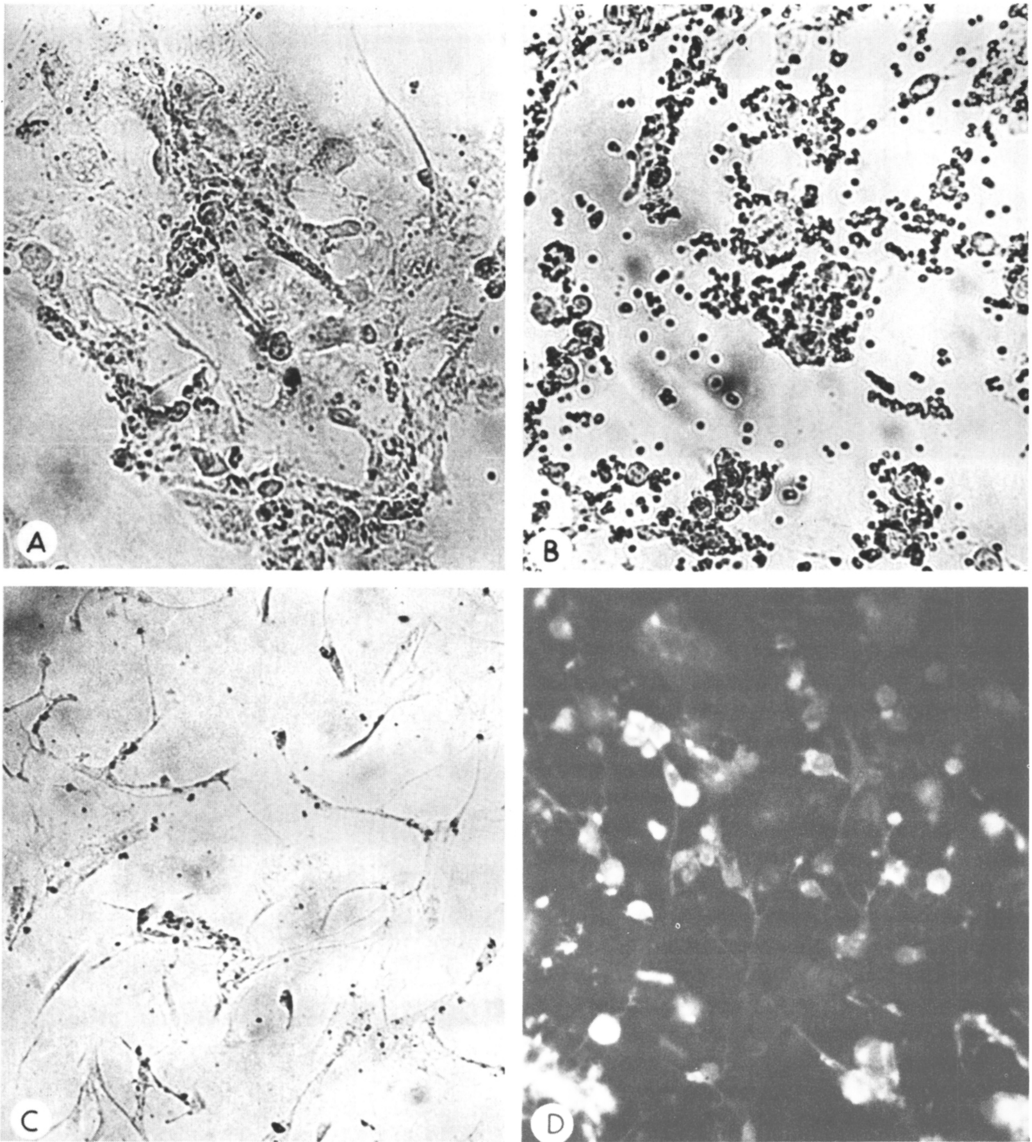

FIG. 1. Infected cells incubated successively with rabbit anti-sheep red blood cell IgG and a 0.5% suspension of sheep red blood cells. 70× (A) VZV-infected Flow 5000 fibroblasts; (B) HSV-infected Flow 5000 fibroblasts; and (C) VZV-infected M-622-CG brain cells. (D) VZV-infected M-622-CG cell fluorescence after successive incubations with human convalescent zoster serum and fluoroscein isothiocyanate-conjugated goat anti-human IgG. 88×

(Fig. 1C). Specific VZV antigens, as expected, were present as shown by indirect immunofluorescence (Fig. 1D).

Virus-induced Fc receptors were also sought using radioiodinated heat-aggregated IgG and $F(ab')_2$ fragments. Virus-infected Flow 5000 fibroblasts were incubated with the radiolabeled immunoglobulin as described under Materials and Methods. The results are given in Table I. Binding of radiolabeled immunoglobulin is more sensitive than sheep cell hemadsorption

for the detection of Fc receptors but is harder to interpret because of the difficulty in adequately washing the cells (particularly damaged cells). Specific binding can be overlooked because of the high background. The results show that human IgG binds to HSV-infected cells to a five fold greater extent than to mock-infected controls or VZV-infected cells. Binding to HSV-infected cells does not disappear when $F(ab')_2$ fragments are used. This is presumably due to specific anti-HSV antibodies contained in the $F(ab')_2$ fragment population. These preparations were commercially purchased and may not be derived from the same immunoglobulin pool. Similar problems are possible with anti-VZV antibodies though the titers are unlikely to be as high. Consequently the experiment was repeated with rabbit IgG and $F(ab')_2$ fragments presumably lacking specific viral antibodies. With these reagents, binding of IgG occurred predominately to HSV-infected cells and not to VZV-infected cells. Binding of the $F(ab')_2$ fragments to VZV- and HSV-infected cells was essentially identical.

Discussion. These studies indicate that VZV, unlike HSV or CMV, does not induce Fc receptors on the surface of infected cells. Such receptors were easily detected by both erythrocyte hemadsorption and radiolabeled immunoglobulin binding on HSV-infected but not VZV-infected cells. The problem of Fc receptors confusing immunologic tests for VZV-specific antigens can thus be safely ignored. The role of Fc receptors in VZV latency is another matter, however. Although we found no evidence of Fc receptors in VZV-infected human brain cells, these cells may not approximate what occurs in the dorsal root ganglion, the presumed site of VZV latency (8). Nevertheless, at the present time, receptors for the Fc portion of IgG do not seem to be important in VZV latency regardless of any role they may have in HSV infection.

Ogata and Shigeta (16) recently reported similar studies with VZV-infected cells. They were able to demonstrate Fc receptors on various cells infected with several VZV strains. They used similar methods to those employed in our study though they documented IgG binding by immunofluorescence rather than radiolabel. Their strongest binding was seen in infected HeLa cells but binding was found in diploid fibroblasts as well. Neither cell type was positive in our hands. These investigators used extraordinarily large amounts of IgG to demonstrate binding with immunofluorescence but this should not have affected the antibody-coated sheep cell hemadsorption. It is also unlikely that our methods were less sensitive since HSV-infected cells, our control cells, were uniformly positive. We were also able to detect Fc receptors on VZV-transformed hamster cells by similar techniques (17). The discrepancy must be put down to cell or virus variation until more information is available. All three of Ogata and Shigeta's VZV strains were derived from patients with herpes zoster rather than varicella but this should not account for the different results as the virus causing each is supposedly identical (18).

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TABLE I. ^{125}I IMMUNOGLOBULIN BINDING

Immunoglobulin	Control cells	HSV-infected cells	VZV-infected cells
Experiment 1			
Human IgG	6494 (0.13)[a]	33,814 (0.69)	6982 (0.14)
Human IgG $F(ab')_2$	57 (0.006)	2667 (0.27)	763 (0.08)
Experiment 2			
Human IgG	1833 (0.04)	9751 (0.20)	1853 (0.04)
Human IgG $F(ab')_2$	160 (0.02)	999 (0.10)	479 (0.05)
Rabbit IgG	98 (0.04)	1184 (0.52)	247 (0.11)
Rabbit IgG $F(ab')_2$	1758 (0.12)	4981 (0.35)	5336 (0.38)

[a] Average cpm bound (% of total cpm added).

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