JOURNAL OF VIROLOGY, Oct. 1985, p. 333–336
0022-538X/85/100333-04$02.00/0

Vol. 56, No. 1

Cross-Reactivity Between Herpes Simplex Virus Glycoprotein B and a 63,000-Dalton Varicella-Zoster Virus Envelope Glycoprotein

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Received 1 April 1985/Accepted 17 June 1985

Cross-reactive monoclonal antibodies recognizing both herpes simplex virus (HSV) glycoprotein B and a major 63,000-dalton varicella-zoster virus (VZV) envelope glycoprotein were isolated and found to neutralize VZV infection in vitro. None of the other VZV glycoproteins was recognized by any polyclonal anti-HSV serum tested. These results demonstrate that HSV glycoprotein B and the 63,000-dalton VZV glycoprotein share antigenic epitopes and raise the possibility that these two proteins have a similar function in infection.

Herpes simplex virus (HSV) and varicella-zoster virus (VZV) are alpha-herpesviruses which cause distinct diseases in humans (17, 37). Primary HSV and VZV infections are very common worldwide, cause considerable morbidity, and under certain circumstances can be life-threatening. As a consequence of the significant health problems resulting from HSV and VZV infections, there is considerable interest in developing effective vaccines to both viruses. We and others have been analyzing the virion structural proteins of VZV and have recently identified the major VZV envelope glycoproteins (2a, 3–5, 11, 19, 34). Further, we have shown that these glycoproteins are present in vivo in vesicular material from varicella and zoster patients. Previous serological evidence has suggested that HSV and VZV share antigenic determinants (25, 33), and the VZV genome has been shown to contain regions of homology to the HSV-1 and HSV-2 genomes (1). Here we report that the envelope glycoproteins HSV gB and VZV gp63 share common antigenic determinants and that cross-reactive polyclonal and monoclonal antibodies can neutralize VZV infection. These findings suggest a common evolutionary ancestor and conserved function for these glycoproteins and have implications for the development of vaccines against HSV and VZV.

Cross-reactive polyclonal anti-HSV sera. To examine whether or not anti-sera to HSV recognize VZV glycoproteins, high-titer rabbit antisera to HSV-1(KOS) and HSV-2(186) were obtained from P. A. Schaffer, Boston, Mass. The characterization of these antisera has been described previously (20). Detergent extracts of [^{3}H]glucosamine-labeled, VZV-infected human foreskin fibroblast cells (350Q) were prepared and subjected to immunoprecipitation as described elsewhere (2a). The immune complexes were isolated with Formalin-fixed, protein A-bearing *Staphylococcus aureus* and washed by a modification of Kessler's procedure (12, 31). Electrophoretic analysis of the immunoprecipitates (Fig. 1a) showed that both antisera recognize major and minor VZV glycoproteins of 63,000 and 125,000 daltons, respectively. (The 125,000-dalton protein is faint in lane 2, but is clearly present upon longer exposure.) These proteins were also immunoprecipitated by an independently derived rabbit antiserum to HSV-1(KOS) provided by J. Glorioso, Ann Arbor, Mich. (data not shown).

To determine whether or not these proteins were the 63,000-dalton VZV envelope glycoprotein (gp63) and its 125,000-dalton precursor (gp125) (19, 34; C. M. Edson, S. C. Hubbard, B. A. Hosler, J. F. Towbin, D. J. Waters, and D. A. Thorley-Lawson, manuscript in preparation), preclearing experiments were carried out with monoclonal antibodies to each of the VZV glycoproteins. The rabbit antiserum to HSV-1(KOS) was used to immunoprecipitate extracts of radiolabeled, VZV-infected cells which were first precleared with a monoclonal antibody to gp63/gp125. Electrophoretic analysis of the immunoprecipitates (Fig. 1b) established that the HSV-1(KOS) antiserum recognized VZV gp63/gp125. Similar results were obtained with the HSV-2(186) antiserum, and neither immunoprecipitation profile was affected by preclearing with monoclonal antibodies to the other VZV envelope glycoproteins gp118 and gp92/gp59/gp47 (data not shown).

Isolation of cross-reactive monoclonal antibodies. Recently, polyclonal antisera to HSV and VZV were used to immunoprecipitate potential cross-reactive proteins, but the identity of these proteins was not determined (27). We tested a variety of monoclonal and monospecific polyclonal antibodies to HSV and VZV glycoproteins and none exhibited any cross-reactivity. Since we were unable to identify the relevant cross-reactive HSV envelope glycoprotein by using existing antisera, we decided to isolate cross-reactive monoclonal antibodies. Four- to 6-week-old BALB/c mice were immunized subcutaneously with 0.5 ml of UV-inactivated HSV-1(KOS) (8×10^8 PFU/ml before inactivation; kindly provided by P. A. Schaffer) in an equal volume of complete Freund adjuvant. The mice were rested for 2 months until the primary immune response subsided and then injected intravenously with 200 μg of partially purified VZV in phosphate-buffered saline. Four days later, spleen cells were prepared and fused to P3 × 63Ag8.653 myeloma cells (9) according to the procedure of Kohler and Milstein (13). We reasoned that under this protocol the primary immune response to VZV would be minimized while selective stimulation of those memory cells specific for cross-reactive epitopes should occur. Serum from a mouse immunized in this manner immunoprecipitated only gp63/gp125 and no other VZV glycoproteins from VZV-infected cells (data not

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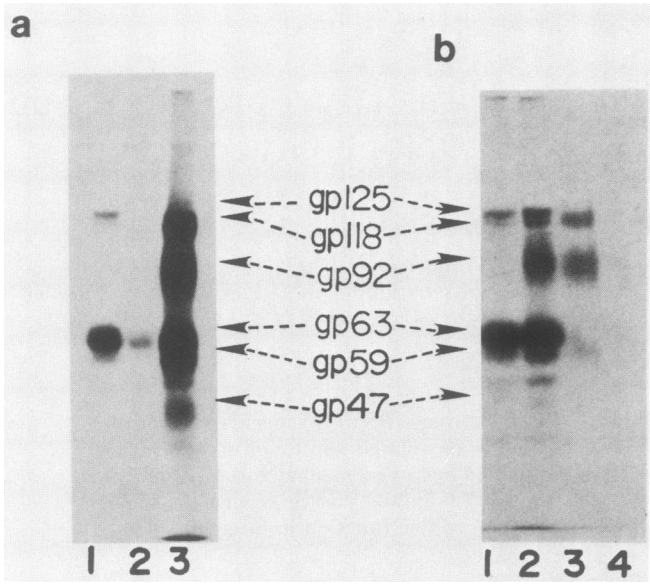

FIG. 1. VZV glycoproteins recognized by polyclonal antisera to HSV-1 and HSV-2. (a) [^{3}H]glucosamine-labeled VZV glycoproteins were immunoprecipitated with a rabbit anti-HSV-1(KOS) antiserum (lane 1), a rabbit anti-HSV-2(186) antiserum (lane 2), or a mouse anti-VZV antiserum (lane 3). (b) Extracts containing [^{3}H]glucosamine-labeled VZV glycoproteins were precleared of VZV gp63/gp125 and then immunoprecipitated with the HSV-1(KOS) antiserum. Lanes 1 and 2, No preclearing, then immunoprecipitation with the rabbit anti-HSV-1(KOS) antiserum (lane 1) or the mouse anti-VZV antiserum (lane 2); lanes 3 to 4, precleared of VZV gp63/gp125, then immunoprecipitation with the mouse anti-VZV antiserum (lane 3) or the rabbit anti-HSV-1(KOS) antiserum (lane 4). The polyclonal mouse antiserum to VZV and the monoclonal antibodies used to preclear VZV gp63/gp125, 1.CE.3D4, and 1.CE.5C3, were prepared in this laboratory. The samples were analyzed by SDS-polyacrylamide gel electrophoresis on 8% acrylamide–0.21% *N,N'*-methylenebisacrylamide gels, treated with En^{3}Hance (New England Nuclear Corp.), dried, and autoradiographed.

shown), a finding which agrees with the specificity observed for the rabbit antisera to HSV. Such a fusion was screened by immunoprecipitation of radiolabeled VZV proteins, and three hybridoma cell lines were isolated which secreted antibody recognizing gp63/gp125 (Fig. 2a). By indirect immunofluorescence, all three antibodies recognized both VZV- and HSV-infected cells (data not shown).

When immunoprecipitation was carried out on radiolabeled HSV-1(KOS) glycoproteins which had been partially purified by lentil lectin affinity chromatography as described previously (22), the monoclonal antibodies all recognized an HSV glycoprotein of about 125,000 daltons (data not shown). To distinguish between HSV glycoproteins gB and gC, both of which have molecular weights in this range, immunoprecipitation experiments were performed on HSV glycoprotein preparations which were precleared with monoclonal antibodies to gB and gC. The HSV monoclonal antibodies used to preclear HSV gB (a pool of 3S, 24S, and 33S) and HSV gC (a pool of 5S and 19S) were obtained from M. Zweig, Bethesda, Md., and have been described previously (28). Electrophoretic analysis of the immunoprecipitates (Fig. 2b) established that the cross-reactive monoclonal antibodies recognized HSV gB. We conclude, therefore, that HSV gB and VZV gp63/125 share antigenic epitopes.

Neutralization of VZV by cross-reactive antibodies. We next evaluated the ability of the cross-reactive antibodies to neutralize VZV and HSV infection in vitro. HSV neutralization assays were carried out on Vero cells as described previously (20) with the following modifications: serial twofold dilutions of the antibodies were incubated with virus at 32°C for 60 min in the presence or absence of 8 U of guinea pig complement (Flow Laboratories, Inc.). Duplicate wells of cells were incubated with virus for 1 h at 37°C followed by an overlay of 2% methylcellulose. VZV neutralization assays were carried out on human foreskin fibroblast cells (350Q) as described elsewhere (2a). For both viruses, the neutralizing antibody titers are expressed as the reciprocal of the dilution of antibody producing a 50% reduction in plaque number. 6.CE.1A2 secretes immunoglobulin G subclass 2a antibody and 6.C.E.9D6 secretes immunoglobulin G subclass 3 antibody, while the antibody isotype for 6.C.E.10E9 has not been determined. We found that the rabbit antiserum to HSV-1(KOS) neutralized VZV infection (Table 1). Furthermore, two of the cross-reactive monoclonal antibodies, 6.CE.1A2 and 6.CE.10E9, also showed complement-dependent neutralization of VZV infection (Table 1). The neutralization is one way since the monoclonal antibodies do not neutralize HSV infection. These results are in contrast to previous studies in which cross-neutralization between HSV and VZV has not been observed (26, 27, 30).

Cross-neutralization has also been reported with antisera that recognize both HSV-1 gB and similar glycoproteins from alpha-herpesviruses of two other species, bovid herpesvirus 2 (BHV-2) and herpes simiae (B-virus) (15, 16, 18). Equine herpesvirus type 1 contains similar cross-reactive material but cross-neutralization with HSV has not been observed (29). Recently, it has been shown that Epstein-Barr virus, a human gamma-herpesvirus, encodes a protein whose amino acid sequence is highly homologous to that of HSV-1(KOS) gB (B. G. Barrell, personal communication). These results suggest a common evolutionary ancestor for these glycoproteins, and it will be of interest to determine whether or not a similar glycoprotein is present in all classes of herpesviruses.

The nature of the cross-reactive epitopes is unknown, but Davison and Wilkie have recently demonstrated that regions of the VZV genome are homologous with the HSV-1 and HSV-2 genomes (1). If the genes for HSV gB and VZV gp63/gp125 correspond to one of these homologous regions, the proteins may share primary sequence epitopes. However, a polyclonal rabbit antiserum to purified, sodium dodecyl sulfate (SDS)-denatured HSV-1(KOS) gB (from R. Courtney, Knoxville, Tenn.) did not recognize native or denatured VZV gp63/gp125, suggesting that at least some of the cross-reactive epitopes present on HSV gB are conformational.

Interestingly, gB and gp63/gp125 share a biochemical feature which is also related to protein conformation. A number of the asparagine-linked oligosaccharides present on mature forms of both viral proteins can be cleaved by endo-β-*N*-acetylglucosaminidase H (endo H) (2a, 8, 35). (In this regard, it would be interesting to examine the endo H sensitivity of the relevant BHV-2 and B-virus glycoproteins.) Recent work with other glycoproteins has suggested that endo H-sensitive glycans occupy sterically inaccessible sites on protein surfaces and are thus unavailable to the Golgi processing enzymes which would otherwise render them endo H resistant (6, 7, 10, 32, 36). In the case of influenza virus hemagglutinin, for example, endo H-sensitive glycans occur in regions where different domains of the protein come in contact, or where it self-associates (10). An

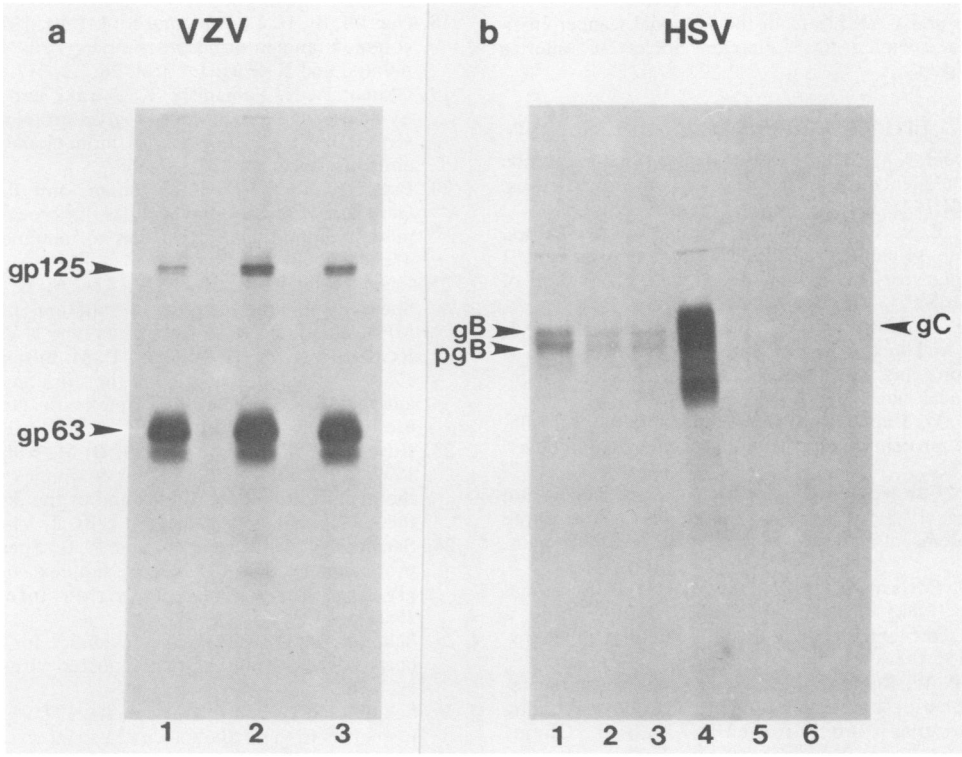

FIG. 2. VZV and HSV glycoproteins recognized by the cross-reactive monoclonal antibodies. (a) [^{3}H]glucosamine-labeled VZV glycoproteins immunoprecipitated by the three cross-reactive monoclonal antibodies and analyzed by 8% SDS-polyacrylamide gel electrophoresis. Lane 1, 6.CE.1A2; lane 2, 6.CE.9D6; lane 3, 6.CE.10E9. (b) Samples of [^{35}S]methionine-labeled HSV-1(KOS) glycoproteins were precleared of HSV gB or HSV gC and then immunoprecipitated with two of the cross-reactive monoclonal antibodies. Preclearing of HSV gB and HSV gC was complete since additional immunoprecipitation gave no detectable bands on SDS-polyacrylamide gel electrophoresis (not shown). Lanes 1 to 3, Preclearing with anti-HSV gC antibodies, then immunoprecipitation with anti-HSV gB monoclonal antibodies (lane 1), 6.CE.1A2 (lane 2), or 6.CE.10E9 (lane 3); lanes 4 to 6, preclearing with anti-HSV gB antibodies, then immunoprecipitation with anti-HSV gC monoclonal antibodies (lane 4), 6.CE.1A2 (lane 5), or 6.CE.10E9 (lane 6). For radiolabeling HSV glycoproteins with [^{35}S]methionine, monolayers of human epidermoid carcinoma cells (HEp-2) in 100-mm culture dishes were infected at 37°C with HSV-1(KOS) at a multiplicity of infection of 20 to 30 PFU per cell. After 1 h at 37°C, the inocula were removed and replaced with RPMI 1640 containing 10% the normal level of methionine and 10% dialyzed fetal calf serum. Four hours later, 1 mCi of L-[^{35}S]methionine (1,237 Ci/mmol; New England Nuclear Corp.) was added and incubation was continued for 20 h. SDS-polyacrylamide gel electrophoresis was carried out on 10% acrylamide–0.29% *N,N'*-diallyltartardiamide gels.

analogous situation may exist with these herpesviruses, since HSV gB forms homodimers (24) and genetic evidence suggests that gB also interacts with other proteins (2, 21, 23).

The retention in gB and gp63 of immunochemical and biochemical features which have conformational bases, together with the fact that HSV and VZV are alphaherpesviruses, suggests that the two proteins share a common biological function in infection. Since gB is thought to be involved in penetration of HSV through the target cell plasma membrane (14, 24), gp63 may serve a similar function in VZV infection. Viral entry could result from the direct action of the proteins themselves or from specific interaction with other host or viral membrane components to promote membrane fusion.

Finally, with vaccines which contain either HSV gB or VZV gp63, the potential clearly exists for cross-reactive immune responses which could confer partial immunity to the heterologous virus. Since the consequences of such responses are not known, it will be important to monitor immunity to both HSV and VZV when evaluating vaccines containing either glycoprotein.

TABLE 1. Neutralization of HSV and VZV infection by cross-reactive antibodies

Antibody	Neutralization titer			
	HSV		VSV	
	+ C	− C	+ C	− C
Polyclonal				
Prebleed	ND[a]	ND	<1:2	ND
Rabbit anti-HSV-1 (KOS)	1:1,000–2,000[b]	ND	1:20	ND
Monoclonal				
6.CE.1A2	<1:2	ND	1:160	<1:20
6.CE.9D6	<1:2	ND	<1:10	<1:20
6.CE.10E9	<1:2	ND	1:160	<1:20

[a] ND, Not determined.
[b] From reference 20.

We thank P. A. Schaffer, B. A. Pancake, S. C. Hubbard, and H. Wortis for helpful discussions, P. G. Spear, G. H. Cohen, J. Glorioso, M. Zweig, R. Courtney, J. Oakes, and R. Ellis for antisera, and B. Barrell for communicating his unpublished data.

This work was supported by Public Health Service grants AI15310 and AI21674 from the National Institute for Allergy and

Infectious Diseases and CA28737 from the National Cancer Institute. R.A.R. was the recipient of a Leukemia Society of America postdoctoral fellowship.

LITERATURE CITED

1. **Davison, A. J., and N. M. Wilkie.** 1983. Location and orientation of homologous sequences in the genomes of five herpesviruses. J. Gen. Virol. **64**:1927–1942.
2. **DeLuca, N., D. Bzik, V. C. Bond, S. Person, and W. Snipes.** 1982. Nucleotide sequences of herpes simplex virus type 1 (HSV-1) affecting virus entry, cell fusion, and production of glycoprotein gB (VP7). Virology **122**:411–423.

2a. **Edson, C. M., B. A. Hosler, C. A. Poodry, R. T. Schooley, D.J. Waters, and D. A. Thorley-Lawson.** 1985. Varicella-zoster virus envelope glycoproteins: biochemical characterization and identification in clinical material. Virology **145**:62–71.

3. **Forghani, B., K. W. Dupuis, and N. J. Schmidt.** 1984. Varicella-zoster viral glycoproteins analyzed with monoclonal antibodies. J. Virol. **52**:55–62.
4. **Grose, C., D. P. Edwards, W. F. Friedrichs, K. A. Weigle, and W. L. McGuire.** 1983. Monoclonal antibodies against three major glycoproteins of varicella-zoster virus. Infect. Immun. **40**:381–388.
5. **Grose, C., D. P. Edwards, K. A. Weigle, W. F. Friedrichs, and W. L. McGuire.** 1984. Varicella-zoster virus-specific gp140: a highly immunogenic and disulfide-linked structural glycoprotein. Virology **132**:138–146.
6. **Hsieh, P., and P. W. Robbins.** 1984. Regulation of asparagine-linked oligosaccharide processing. Oligosaccharide processing in *Aedes albopictus* mosquito cells. J. Biol. Chem. **259**:2375–2382.
7. **Hsieh, P., M. R. Rosner, and P. W. Robbins.** 1983. Selective cleavage by endo-β-N-acetylglucosaminidase H at individual glycosylation sites of Sindbis virion envelope glycoproteins. J. Biol. Chem. **258**:2555–2561.
8. **Johnson, D. C., and P. G. Spear.** 1983. O-linked oligosaccharides are acquired by herpes simplex virus glycoproteins in the Golgi apparatus. Cell **32**:987–997.
9. **Kearney, J. F., A. Radbruch, B. Liesegang, and K. Rajewsky.** 1979. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. J. Immunol. **123**:1548–1550.
10. **Keil, W., H. Niemann, R. T. Schwarz, and H.-D. Klenk.** 1984. Carbohydrates of influenza virus. V. Oligosaccharides attached to individual glycosylation sites of the hemagglutinin of fowl plague virus. Virology **133**:77–91.
11. **Keller, P. M., B. J. Neff, and R. W. Ellis.** 1984. Three major glycoprotein genes of varicella-zoster virus whose products have neutralization epitopes. J. Virol. **52**:293–297.
12. **Kessler, S.** 1975. Rapid isolation of antigens from cells with a staphyloccal protein A antibody absorbent. J. Immunol. **115**:1617–1624.
13. **Kohler, G., and C. Milstein.** 1975. Continuous culture of fused cells secreting antibody of predefined specificity. Nature (London) **256**:495–497.
14. **Little, S. P., J. T. Jofre, R. J. Courtney, and P. A. Schaffer.** 1981. A virion associated glycoprotein essential for infectivity of herpes simplex virus type 1. Virology **115**:149–160.
15. **Ludwig, H.** 1983. Bovine herpesviruses, p. 135–214. *In* B. Roizman (ed.), The herpesviruses, vol. 2. Plenum Press, New York.
16. **Ludwig, H., G. Pauli, H. Gelderblom, G. Darai, H.-G. Koch, R. M. Flugel, B. Norrild, and M. D. Daniel.** 1983. B. virus (*Herpesvirus simiae*), p. 385–428. *In* B. Roizman (ed.), The herpesviruses, vol. 2. Plenum Press, New York.
17. **Nahmias, A. J., J. Dannenbarger, C. Wickliffe, and J. Muther.** 1981. Clinical aspects of infection with herpes simplex viruses 1 and 2, p. 3–9. *In* A. J. Nahmias, W. R. Dowdle, and R. E. Schinazi (ed.), The human herpesviruses. Elsevier, New York.
18. **Norrild, B., H. Ludwig, and R. J. Rott.** 1978. Identification of a common antigen of herpes simplex virus, bovine herpes mammillitis, and B virus. J. Virol. **26**:712–717.
19. **Okuno, T., K. Yamanishi, K. Shiraki, and M. Takahashi.** 1983. Synthesis and processing of glycoproteins of varicella-zoster virus (VZV) as studied with monoclonal antibodies to VZV antigens. Virology **129**:357–368.
20. **Pancake, B. A., D. P. Aschman, and P. A. Schaffer.** 1983. Genetic and phenotypic analysis of herpes simplex virus type 1 mutants conditionally resistant to immune cytolysis. J. Virol. **47**:568–585.
21. **Pogue-Geile, K. L., G. T.-Y. Lee, S. K. Shapiro, and P. G. Spear.** 1984. Fine mapping of mutations in the fusion-inducing MP strain of herpes simplex virus type 1. Virology **136**:100–109.
22. **Respess, R. A., B. A. Pancake, C. M. Edson, and P. A. Schaffer.** 1984. A rapid procedure for the enrichment of undenatured, antigenically active herpes simplex virus glycoproteins. J. Virol. Methods **8**:27–45.
23. **Ruyechan, T. W., L. S. Morse, D. M. Knipe, and B. Roizman.** 1979. Molecular genetics of herpes simplex virus. II. Mapping of the major viral glycoproteins and of the genetic loci specifying the social behavior of infected cells. J. Virol. **29**:677–697.
24. **Sarmiento, M., M. Haffey, and P. G. Spear.** 1979. Membrane proteins specified by herpes simplex viruses. III. Role of glycoprotein VP7(B_2) in virion infectivity. J. Virol. **29**:1149–1158.
25. **Schmidt, N. J.** 1982. Further evidence for common antigens in herpes simplex and varicella-zoster viruses. J. Med. Virol. **9**:27–36.
26. **Schmidt, N. J., and E. H. Lennette.** 1975. Neutralizing antibody responses to varicella-zoster virus. Infect. Immun. **12**:606–613.
27. **Shiraki, K., T. Okuno, K. Yamanishi, and M. Takahashi.** 1982. Polypeptides of varicella-zoster virus (VZV) and immunological relationship of VZV and herpes simplex virus (HSV). J. Gen. Virol. **61**:255–269.
28. **Showalter, S. D., M. Zweig, and B. Hampar.** 1981. Monoclonal antibodies to herpes simplex virus type 1 proteins, including the immediate-early protein ICP 4. Infect. Immun. **34**:684–692.
29. **Snowden, B. W., P. R. Kinchington, K. L. Powell, and I. W. Halliburton.** 1985. Antigenic and biochemical analysis of gB of herpes simplex virus type 1 and type 2 and of cross-reacting glycoproteins induced by bovine mammillitis virus and equine herpesvirus type 1. J. Gen. Virol. **66**:231–247.
30. **Svedmyr, A.** 1965. Varicella virus in HeLa cells. Arch. Virusforsch. **17**:495–503.
31. **Thorley-Lawson, D. A., and C. M. Edson.** 1979. Polypeptides of the Epstein-Barr virus membrane antigen complex. J. Virol. **32**:458–467.
32. **Trimble, R. B., F. Maley, and F. K. Chu.** 1983. Glycoprotein biosynthesis in yeast. Protein conformation affects processing of high mannose oligosaccharides on carboxypeptidase Y and invertase. J. Biol. Chem. **258**:2562–2567.
33. **Trlifajova, J., J. Sourek, and M. Ryba.** 1971. Preparation of concentrated intracellular precipitating antigens of varicella-zoster and herpes simplex viruses and results obtained with them in the gel precipitation reaction. Acta Virol. **15**:293–300.
34. **Vafai, A., Z. Wroblewska, M. Wellish, M. Green, and D. J. Gilden.** 1984. Analysis of three late varicella-zoster virus proteins, a 125,000-molecular-weight protein and gp1 and gp3. J. Virol. **52**:953–959.
35. **Wenske, E. A., M. W. Bratton, and R. J. Courtney.** 1982. Endo-β-*N*-acetylglucosaminidase H sensitivity of precursors to herpes simplex virus type 1 glycoproteins gB and gC. J. Virol. **44**:241–248.
36. **Wilson, I. A., J. J. Skehel, and D. C. Wiley.** 1981. Structure of the haemagglutinin membrane glycoprotein of influenza virus at 3Å resolution. Nature (London) **289**:366–373.
37. **Zaia, J. A.** 1981. Clinical spectrum of varicella-zoster virus infection, p. 10–19. *In* A. J. Nahmias, W. R. Dowdle, and R. E. Schinazi (ed.), The human herpesviruses. Elsevier, New York.