Affinity-purified Varicella-Zoster Virus Glycoprotein gp1/gp3 Stimulates the Production of Neutralizing Antibody

By ZOFIA WROBLEWSKA, DONALD GILDEN, MARGUERITE GREEN, MARY DEVLIN AND ABBAS VAFAI

Multiple Sclerosis Research Center, The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, Pennsylvania 19104, Department of Microbiology and Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, U.S.A.

(Accepted 8 May 1985)

SUMMARY

Varicella-zoster virus glycoprotein gp1/gp3 was purified by affinity chromatography using anti-gp1/gp3 monoclonal antibody 19.1 linked to CNBr-activated Sepharose CL-4B. Rabbits immunized with purified glycoprotein gp1/gp3 developed monospecific neutralizing antibody.

Varicella-zoster virus (VZV) induces at least seven glycosylated proteins in infected cells, four of which are major glycoproteins with molecular weights of 115K to 120K (gp1), 80K to 100K (gp2), 62K to 64K (gp3) and 55K (gp5) (Asano & Takahashi, 1979; Grose, 1980; Grose et al., 1981, 1983, 1984; Grose & Friedrichs, 1982; Shiraki & Takahashi, 1982; Okuno et al., 1983; Forghani et al., 1984; Friedrichs & Grose, 1984; Keller et al., 1984).

We have previously described the production and characterization of a monoclonal antibody (MAb 19.1) which recognizes an epitope on the late glycoproteins gp1 and gp3 (Vafai et al., 1984). This antibody has virus-neutralizing activity, reacts with membrane antigens of infected cells as well as with the virion envelope and immunoprecipitates gp1 and gp3. In pulse-chase experiments, only gp1 was detected in pulse-labelled infected cells, whereas both gp1 and gp3 were detected during the chase period (Okuno et al., 1983; Vafai et al., 1984). Immunoprecipitation of purified whole virions and nucleocapsids using MAb 19.1 revealed only gp3 in the virions. These data suggested that gp3 may be the mature form of a viral glycoprotein that is a cleavage product of gp1 (Okuno et al., 1983; Vafai et al., 1984), and it is possible that gp3 plays a role in the interaction of the virion with the host cell surface.

The purpose of this study was to purify VZV glycoproteins gp1/gp3 using MAb 19.1 as an immunoadsorbent and to apply the purified proteins as an immunogen to stimulate the production of virus-neutralizing antibodies in rabbits. This is the first successful attempt at purification of immunogenic VZV glycoproteins.

The MB strain of VZV, originally isolated from a patient with shingles and characterized in this laboratory, was propagated and titrated in BSC-1 monkey cells as previously described (Gilden et al., 1978).

Immunoglobulin G (19.1 IgG) was purified from mouse ascitic fluid containing MAb 19.1 using a Protein A–Sepharose CL-4B column (Pharmacia). Purified 19.1 IgG was tested by immunofluorescence (IF) for binding to VZV-specific antigens on unfixed infected BSC-1 cells. As shown in Fig. 1 (a), fluorescence on the surface membrane of infected cells was comparable to that seen with ascitic fluid containing MAb (Fig. 1 b).

The gp1/gp3 proteins were affinity-purified; the average yield of purified proteins was 40 μg per 2 × 10⁹ VZV-infected cells. SDS-PAGE analysis of the purified proteins resulted in the detection of gp1/gp3 (Fig. 2, lane 1) and two other proteins of apparent mol. wt. of 60K and 45K. The 60K protein could be the cleavage product of the 62K (gp3) protein because it reacts with...
Short communication

Fig. 1. Indirect IF staining of VZV-infected cell membranes with purified 19.1 IgG (a), MAb 19.1 ascitic fluid (b), and rabbit anti-gp1/gp3 serum (c). Cells grown on coverslips were infected with VZV and 24 to 48 h post-infection unfixed cells were incubated with each antibody preparation (diluted 1:10) followed by FITC-conjugated goat anti-mouse or anti-rabbit IgG (Cappel Laboratories) diluted 1:10 (Gilden et al., 1978). No fluorescence was seen after application of antibody to uninfected cells. x 300.

IgG was purified as follows. Protein A-Sepharose CL-4B was rehydrated in 0.1 M-sodium phosphate buffer pH 7.0, put in the column and washed with 0.1 M-phosphate buffer pH 8.0. The ascitic fluid containing MAb 19.1 was diluted 1:2 in 0.1 M-phosphate buffer pH 8.0, and applied to the column at room temperature. Unbound proteins were removed by extensive washing with 0.1 M-phosphate buffer pH 8.0. IgG was eluted with 25 ml 0.1 M-sodium citrate pH 4.0, and 2.5 ml fractions were collected, neutralized with 1 M-Tris-HCl pH 8.0; the protein concentration in each was determined by the method of Bramhall et al. (1969). Fractions with the highest protein concentrations were pooled, dialysed against 0.02 M-phosphate buffer pH 7.2, lyophilized overnight, rehydrated in H2O (2 mg/ml) and stored at -70 °C.

human serum, rabbit anti-VZV sera and ascitic fluid containing MAb 19.1 (Fig. 2, lanes 2, 3 and 4). When uninfected cell lysates were precipitated along with VZV-infected cell lysates during the purification procedures, the 45K protein was detected in both preparations by SDS-PAGE (data not shown) indicating that this is a host cell protein which binds to the affinity column.

One additional protein of approx. mol. wt. 90K was also detected in the purified gp1/gp3 preparations and was precipitated with ascitic fluid MAb 19.1 (Fig. 2, lane 4), but not with human or rabbit anti-VZV sera (Fig. 2, lanes 2 and 3). This difference probably reflects the higher concentration of anti-gp1/gp3 antibodies in the ascitic fluid MAb than in human or rabbit anti-VZV sera. Densitometric quantification from autoradiographs (Fig. 2, lane 1) revealed higher concentrations of gp3 than of gp1 in the purified preparation; this could reflect either a greater amount of gp3 in the VZV-infected cell lysate or a higher affinity of 19.1 IgG for gp3 than for gp1.

The immunogenicity of purified gp1/gp3 was then tested in New Zealand rabbits immunized subcutaneously or intramuscularly with purified protein preparations (10 µg) in complete Freund’s adjuvant followed by three weekly injections of gp1/gp3 in incomplete Freund’s adjuvant. Seven days after the last injection, the animals were bled and their sera were tested for the presence of anti-gp1/gp3 antibody by IF, immunoprecipitation and neutralization assays. Fig. 1 (c) illustrates the reactivity of rabbit anti-gp1/gp3 serum with unfixed infected cells by IF.

Fig. 2. Purification of VZV gp1/gp3. The proteins were purified from [35S]methionine-labelled VZV-infected cell lysates according to the method of Eisenberg et al. (1982) with minor modifications. BSC-1 cells (1 x 10⁸) were infected with VZV (Gilden et al., 1978) and at 24 h post-infection, washed with serum-free MEM and overlaid with MEM containing one-tenth the normal concentration of
methionine with 2% FBS and 50 μCi/ml [35S]methionine (sp. act. 1000 Ci/mmol, Amersham) for 24 h at 37 °C. Labelled cells were scraped into the medium and centrifuged at 2000 g for 20 min at 4 °C. The cell pellets were washed with cold phosphate-buffered saline (PBS) and disrupted at 4 °C for 2 h in 4 ml lysis buffer (0.01 M-sodium phosphate pH 7.6, 0.1 M-NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS). The lysates were centrifuged for 20 min at 2000 r.p.m. then the supernatant at 175000 g in a Beckman SW65 rotor for 2 h at 4 °C. The supernatants were used for purification of gp1/gp3. One g CNBr-activated Sepharose 4B beads was swollen in 0.001 N-HCl for 15 min, washed with 200 ml of 0.001 N-HCl, and resuspended in 6 ml coupling buffer (0.1 M-NaHCO3 pH 8.3, 0.5 M-NaCl). Ten ml purified IgG suspension (2 mg/ml in coupling buffer) was added and the mixture was incubated for 2 to 3 h at room temperature with rocking. To block remaining active groups, the beads were resuspended in 10 ml 0.2 M-glycine pH 8.0, rocked for 2 h at room temperature and washed three times with 0.1 M-sodium acetate pH 4.0, 1 M-NaCl followed by 0.1 M-NaHCO3 pH 8.0, 1 M-NaCl. The beads were then resuspended in 10 ml TSN buffer (0.01 M-Tris–HCl pH 7.5, 0.5 M-NaCl, 0.05% NP40, 0.1 mM-PMSF) and applied to the column. The column was washed with 10 vol. TSN buffer, and the VZV-infected cell lysates (from 10⁸ cells) were applied and recycled through the column ten times. The column was washed overnight at 4 °C with 21 TSN buffer to remove unbound proteins. Bound proteins were eluted with 50 ml 3 M-KSCN, 0.1 M-Tris–HCl pH 7.8. The eluate was dialysed against three changes (21 each) of 1 mM-Tris–HCl pH 7.5, 0.01% NP40, 0.01 mM-PMSF, lyophilized overnight, resuspended in 0.1 vol. H2O, and either analysed by SDS–PAGE (lane 1) or immunoprecipitated with human anti-VZV serum (lane 2), rabbit anti-VZV serum (Wroblewska et al., 1982) (lane 3), or ascitic fluid containing MAb 19.1 (lane 4) and analysed in a 10% polyacrylamide gel. For immunoprecipitation, purified gp1/gp3 (4 to 5 μg/sample) were incubated at 4 °C for 20 h with 50 μl human anti-VZV serum, rabbit anti-VZV serum, or ascitic fluid containing MAb 19.1. Finally, 50 μl of a formalin-fixed 10% suspension of Staphylococcus aureus Cowan I strain (Kessler, 1975) was added, and after 2 h at 4 °C, the absorbed immune complexes were washed three times with lysis buffer, resuspended in 50 mM-Tris–HCl pH 7.4, 150 mM-NaCl, 5mM-EDTA and analysed by gel electrophoresis (Laemmli, 1970). Gels were processed for fluorography (En’Hance, New England Nuclear), dried and exposed to Kodak X-Omat AR film at –70 °C.

Fig. 3. Immunoprecipitation of VZV-infected cell lysates using MAb 19.1 (lane 1), purified 19.1 IgG (lane 2) or rabbit anti-gp1/gp3 serum (lane 3). [35S]Methionine-labelled VZV-infected cell lysates were prepared, immunoprecipitated with the antibodies, and analysed by 10% SDS–PAGE under reducing (RC), and non-reducing (NC) conditions in which 2-mercaptoethanol was excluded from the sample buffer. Before immunoprecipitation of virus-specific proteins, 1 ml portions of the supernatants were incubated for 20 h at 4 °C with 10 μl of normal rabbit serum and 50 μl of a 10% S. aureus suspension followed by centrifugation at 2000 g for 20 min.
Immunoprecipitation of \([^{35}S]\)methionine-labelled VZV-infected cell lysates with rabbit anti-gp1/gp3 sera followed by SDS-PAGE revealed a strong band corresponding to gp3 and a faint band for gp1 (Fig. 3). It is possible that the particular immunogen preparation contained a higher concentration of gp3. Alternatively, gp3 might elicit a better immune response than gp1. SDS-PAGE analysis under non-reducing conditions revealed a 130K protein (Fig. 3, lane 3), but not the 62K protein band (gp3), consistent with previous results indicating that gp3 is a disulphide-linked dimer (Grose et al., 1984; Vafai et al., 1984). This dimer form was detected by MAb 19.1 as well as by the rabbit serum prepared against purified proteins.

The rabbit anti-gp1/gp3 sera were also examined for virus neutralizing activity. Neutralization was performed by the constant virus-varying serum technique (Schmidt & Lennette, 1975). Cells (10⁸) were infected with VZV by co-cultivation (Gilden et al., 1978). After 4 days, when infected cultures showed 80 to 90% c.p.e., cells were scraped into the tissue culture medium, centrifuged at 2000 g for 20 min at 4 °C and the cell pellet was resuspended in 4 ml of serum-free MEM. The cell suspension was homogenized (100 strokes) in a Teflon-coated homogenizer to break the cytoplasmic and nuclear membranes. The homogenates were centrifuged at 800 g for 10 min and the supernatants were used for neutralization tests. Aliquots (0.5 ml) containing 100 to 200 p.f.u. were mixed with equal volumes of serial dilutions (1:2 to 1:1024) of rabbit anti-gp1/gp3 or preimmune serum. The mixtures were incubated at 37 °C for 1 h and inoculated into two wells of BSC-1 cells grown in six-well plates. After 3 h adsorption at 37 °C, the inoculum was removed, cells were overlaid with MEM containing 2% foetal bovine serum (FBS) and incubated at 37 °C for 6 to 7 days. Cells were fixed with formaldehyde, stained with cresyl violet and plaques were counted. The results showed that rabbit anti-gp1/gp3 serum diluted 1:2 to 1:32 neutralized virus infectivity by 91 to 94% and 1:1024 diluted serum reduced plaque counts by 60 to 63%, demonstrating positive seroconversion in animals immunized with purified gp1/gp3.

Previously, we showed that gp1/gp3 proteins are expressed on the VZV-infected cell surface, gp3 is found in the envelope of VZV virions and that MAb 19.1 prepared against VZV-induced gp1/gp3 glycoproteins is capable of neutralizing VZV infectivity (Vafai et al., 1984). In the present study, we have shown that purified gp1/gp3 proteins retain immunogenic activity and stimulate the production of neutralizing antibody, suggesting that gp1/gp3 proteins may play a role in the interaction of VZV and the cell surface in the initial stage of viral infection.

We thank R. J. Eisenberg for a critical review of the manuscript, M. Wellish for expert technical assistance, A. Gilden for printing the photographs, M. Hoffman for editorial review, and J. DeRitis for preparing the manuscript. This work was supported by grants NS-11037 from the National Institutes of Health and 894-D4 from the National Multiple Sclerosis Society.

REFERENCES


*(Received 22 January 1985)*