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Recognition of Similar Epitopes on Varicella-Zoster Virus gpI and gpIV by Monoclonal Antibodies

ABBAS VAFAI,[1,2]* ZOFIA WROBLEWSKA,[3] RAVI MAHALINGAM,[1] GARY CABIRAC,[1] MARY WELLISH,[1] MARIA CISCO,[3] AND DONALD GILDEN[1]

Departments of Neurology[1] and Microbiology and Immunology,[2] University of Colorado School of Medicine, Denver, Colorado 80262, and the Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104[3]

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Two monoclonal antibodies, MAb43.2 and MAb79.0, prepared against varicella-zoster virus (VZV) proteins were selected to analyze VZV gpIV and gpI, respectively. MAb43.2 reacted only with cytoplasmic antigens, whereas MAb79.0 recognized both cytoplasmic and membrane antigens in VZV-infected cells. Immunoprecipitation of in vitro translation products with MAb43.2 revealed only proteins encoded by the gpIV gene, whereas MAb79.0 precipitated proteins encoded by the gpIV and gpI genes. Pulse-chase analysis followed by immunoprecipitation of VZV-infected cells indicated reactivity of MAb43.2 with three phosphorylated precursor species of gpIV and reactivity of MAb79.0 with the precursor and mature forms of gpI and gpIV. These results indicated that (i) MAb43.2 and MAb79.0 recognize different epitopes on VZV gpIV, (ii) glycosylation of gpIV ablates recognition by MAb43.2, and (iii) gpIV is phosphorylated. To map the binding site of MAb79.0 on gpI, the pGEM transcription vector, containing the coding region of the gpI gene, was linearized, and three truncated gpI DNA fragments were generated. RNA was transcribed from each truncated fragment by using SP6 RNA polymerase, translated in vitro in a rabbit reticulocyte lysate, and immunoprecipitated with MAb79.0 and human sera. The results revealed the existence of an antibody-binding site within 14 amino acid residues located between residues 109 to 123 on the predicted amino acid sequences of gpI. From the predicted amino acid sequences, 14 residues on gpI (residues 107 to 121) displayed a degree of similarity (36%) to two regions (residues 55 to 69 and 245 to 259) of gp IV. Such similarities may account for the binding of MAb79.0 to both VZV gpI and gpIV.

Varicella-zoster virus (VZV) DNA encodes five glycoproteins, designated gpI, gpII, gpIII, gpIV, and gpV, of which gpI through gpIV are readily detected in infected cells and in VZ virions (5). gpIV and gpI are encoded by genes 67 and 68, respectively, located within the unique short sequences (U_S) of the VZV genome (7, 8, 11). Genes 67 and 68 encode primary translation products of 39 and 70 kilodaltons (kDa) (7, 8, 11) that are further processed into mature forms of 60 (gpIV) and 95 (gpI) kDa, respectively (30, 31, 36). Comparisons of the DNA sequences of U_S regions of VZV genome with those of two other alphaherpesviruses, herpes simplex virus (HSV) and pseudorabies virus (PRV), have identified a degree of conservation in the predicted amino acid sequences of VZV (gpIV and gpI) with HSV (gI and gE) and PRV (gp63 and gI) (6, 24, 27, 34). Such similarities suggest the existence of a yet unidentified common function among these glycoproteins. The HSV gE functions as a receptor for the Fc component of immunoglobulin G (IgG) (2). In addition, HSV gp70 (corresponding to HSV gI) and gE form a complex which binds to IgG (18, 24). However, no Fc-binding activity has been detected for VZV gpI (10), and to date no information is available whether PRV encodes an Fc receptor. Studies on HSV gI and gE and PRV gp63 and gI have shown that these glycoproteins are dispensable for virus replication in culture (23, 24, 28, 32), but PRV gI is involved in the virulence of PRV (3, 23). At present, little is known about the biological function of VZV gpI and gpIV during virus replication; whether these glycoproteins have functions similar to those of HSV and PRV remains to be determined. However, VZV gpI and gpIV appear to be important in the induction of immune response to VZV infection. The most abundant and immunogenic of the VZV glycoproteins, gpI, elicits the formation of complement-dependent neutralizing antibodies and also cell-mediated immunity (1, 12, 16, 17, 20). Monoclonal antibodies (MAbs) produced against gpI neutralize VZV infectivity in the presence of complement and also recognize multiple protein species ranging in size from 45 to 98 kDa (9, 12, 13, 20, 29). Since VZV gene 68 encodes a primary translation product of approximately 70 kDa, which is further processed into a mature form of 95 to 98 kDa (30, 37), the reactivity of these MAbs with other protein species is not clear. Therefore, the present work was designed to further characterize VZV gpI and gpIV by using MAbs and polyclonal antibodies produced against these glycoproteins. Our findings indicated that one MAb recognized the primary translation products encoded by gpI and gpIV genes and precipitated the precursor and mature forms of these two glycoproteins in VZV-infected cells. The binding site of this MAb was shown to be located within 14 residues on the predicted amino acid sequences of gpI. In addition, amino acid sequence similarities were identified between gpI and gpIV which may account for the binding of one MAb to two different glycoproteins. We were also able to identify the previously unidentified phosphorylated species of gpIV and determine that glycosylation of gpIV ablates its recognition by the MAb directed against this protein.

MATERIALS AND METHODS

Cells and virus. VZV was propagated in BSC-1 cells by cocultivation of trypsinized BSC-1 cells and VZV-infected cells at a ratio of 4:1 as described previously (14, 15).

Preparation of MAbs and polyclonal antibodies. MAbs (MAb43.2 and MAb79.0) against VZV proteins were pre-

* Corresponding author.

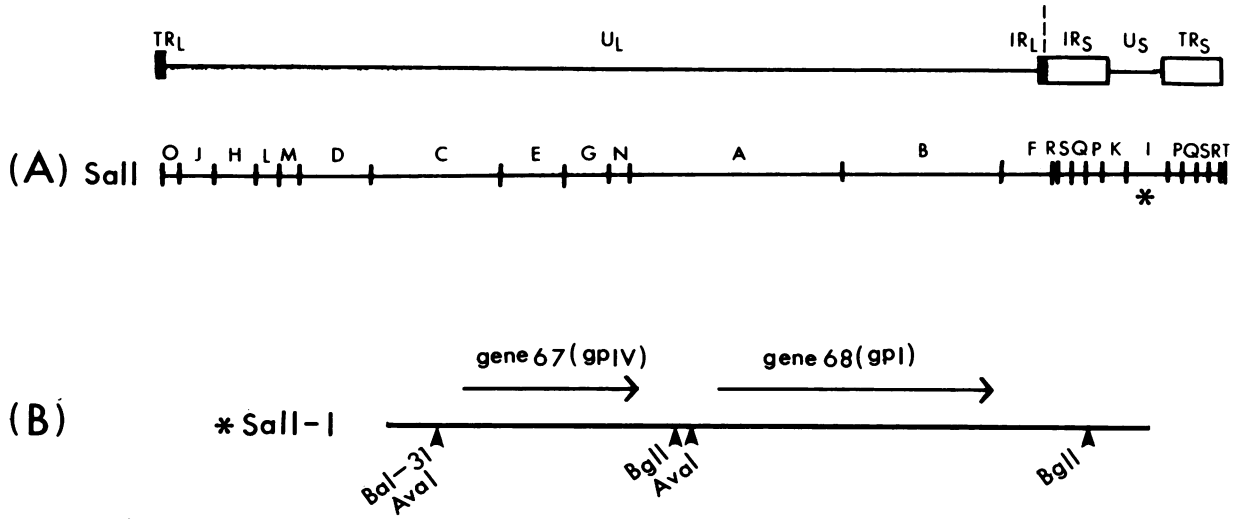

FIG. 1. (Top) Physical map of VZV DNA, which consists of two covalently linked segments, a unique long (U_L) and a unique short (U_S) segment, bounded by inverted repeat sequences (IRs/TRs). An inverted repeat of 88.5 bp also flanks U_L. (A) *Sal*I restriction sites derived from the detailed restriction and DNA sequence analysis of VZV genome (7). (B) VZV *Sal*I-I DNA fragment containing the coding regions of genes 67 (gpIV) and 68 (gpI). The locations of Bal-31 and restriction sites used for subcloning of these genes into pGEM transcription vector are shown.

pared as described previously (37). Briefly, BALB/c mice were immunized subcutaneously with purified viral particles, and splenocytes were fused with SP2/O-Ag14 mouse myeloma cells. Positive hybridomas were identified by an enzyme-linked immunosorbent assay and grown in tissue culture. The isotype of each MAb was determined by immunofluorescence on VZV-infected cells incubated with undiluted culture fluid containing the MAb, followed by incubation with rabbit isotype-specific serum (a gift from W. Gerhard) and finally with a 1:10 dilution of fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG. Polyclonal antibodies against VZV proteins and the in vitro translation products encoded by VZV gene 67 (gpIV) and 68 (gpI) were prepared as described elsewhere (36, 39, 40). Human sera containing high titers of antibodies to VZV glycoproteins were obtained from an 86-year-old female suffering from postherpetic neuralgia.

Immunofluorescence assay. MAb43.2 and MAb79.0 were assayed by indirect immunofluorescence on both unfixed and acetone-fixed VZV-infected cells with a 1:5 dilution of MAb and 1:10 dilution of FITC-conjugated goat anti-mouse IgG (Cappel Laboratories) as described (37).

Cloning of VZV genes 67 (gpIV) and 68 (gpI). The VZV *Sal*I-I DNA fragment (Fig. 1) contains the open reading frames (ORFs) for VZV genes 67 and 68 (7). The recombinant plasmid containing the *Sal*I-I fragment (35) was cleaved with *Sal*I endonuclease, and the DNA fragments were separated on 0.6% agarose gels at 40 V for 16 h at room temperature. The *Sal*I-I fragment was extracted by electroelution (26) and cleaved with *Bgl*I restriction enzyme. The 2.5-kilobase (kb) *Bgl*I fragment containing the ORF of gpI was electroeluted, blunt-ended with T4 DNA polymerase (26), and ligated into the *Sma*I site downstream from the SP6 promoter of the pGEM (Promega Biotec) transcription vector (35). To clone gene 67 (gpIV), the *Sal*I-I fragment was digested with Bal-31 (International Biotechnologies, Inc.) for various time periods according to the manufacturer's instructions. The DNA fragments were extracted with phenol-chloroform, ethanol precipitated, blunt-ended with S1 nuclease and Klenow I DNA polymerase (26), and ligated into the *Sma*I site of the pGEM vector. The nucleotide sequences of the ends of the Bal-31-digested DNA fragments were determined by the GemSeq sequencing system (Promega Biotec) according to the manufacturer's instructions. A recombinant plasmid containing a DNA fragment (3,209 base pairs [bp]) spanning nucleotides 114,385 to 117,594 of the VZV genome (7) was cleaved with *Ava*I restriction enzyme. The *Ava*I DNA fragment (1,375 bp) spanning nucleotides 114,385 to 115,760 of the VZV genome and containing the coding region of gene 67 (gpIV) was extracted by electroelution and cloned at the *Ava*I site of the pGEM vector. To prepare truncated gpI DNA fragments, the recombinant pGEM vector carrying the *Bgl*I fragment was linearized with *Hin*dIII downstream from the DNA insert, and three DNA fragments were generated by digestion of the *Bgl*I fragment with *Cla*I, *Xho*II, and *Bgl*II endonucleases at 322, 372, and 480 nucleotides downstream from the predicted initiation codon (ATG) of the VZV gpI gene, respectively. Truncated DNA fragments were extracted with phenol-chloroform, precipitated with ethanol, and suspended in water (1 μg/2 μl).

In vitro transcription and translation. The recombinant plasmids carrying either VZV gene 67 (gpIV) or gene 68 (gpI) were cleaved with *Hin*dIII restriction enzyme downstream from the DNA insert, and the linearized DNA was used as a template for RNA transcription with SP6 RNA polymerase (Promega Biotec). RNA was transcribed from genes 67, 68, and truncated gpI DNA fragments and translated in vitro, and the in vitro translation (IVT) products were either analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (35) or immunoprecipitated with monoclonal and polyclonal antibodies and analyzed by SDS-PAGE as described below.

Radioactive labeling of VZV-infected cell proteins. VZV-infected cells were labeled with 500 μCi of either [^{3}H] mannose (specific activity, 15 to 25 Ci/mmol; ICN radiochemicals) per ml for 4 h or $^{32}P_i$ (ICN radiochemicals) per ml for 2 h at 37°C. For pulse-chase experiments, infected cells were labeled with 300 μCi of [^{35}S]methionine (specific activity, >1,000 Ci/mmol; New England Nuclear Corp.) per ml for 10 min in the absence or presence of tunicamycin (15 μg/ml).

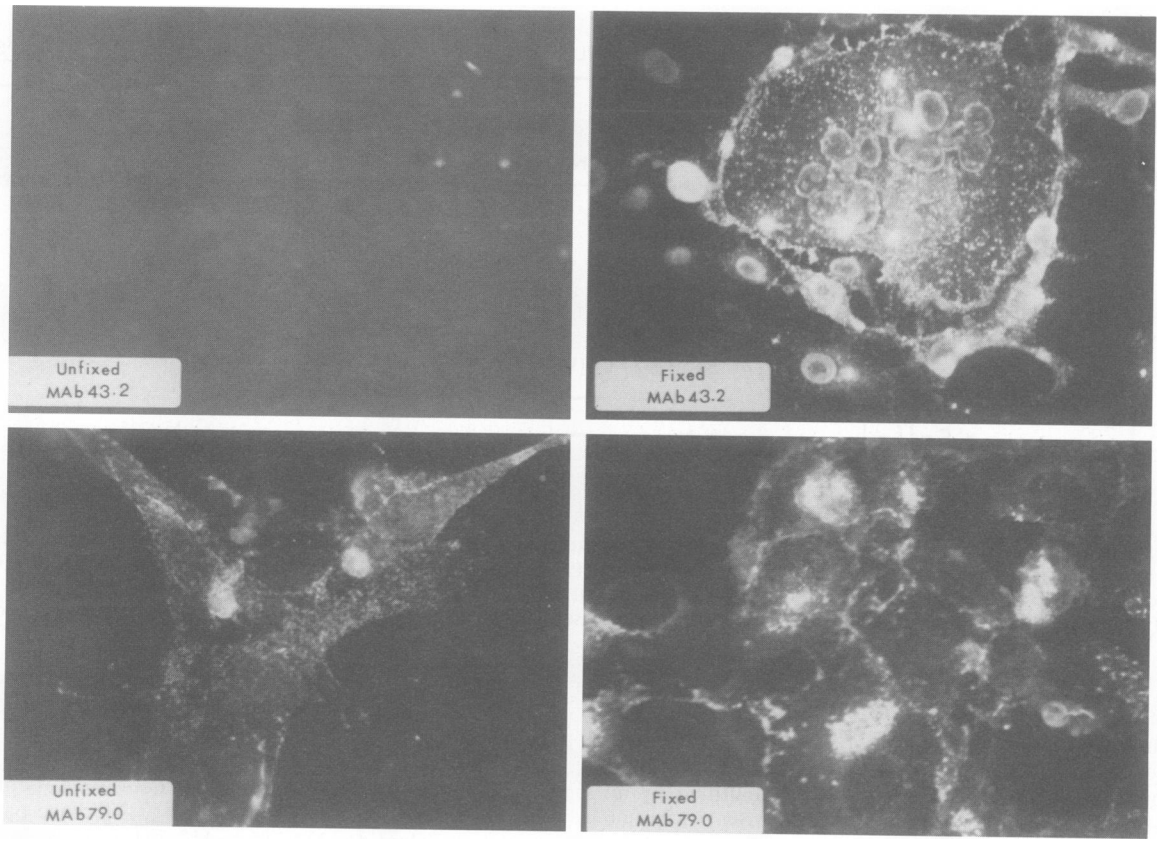

FIG. 2. Immunofluorescence staining of VZV-infected cells with MAb43.2 and MAb79.0. Unfixed and acetone-fixed VZV-infected cells grown on cover slips were reacted with MAbs and FITC-conjugated goat anti-mouse IgG as described in Materials and Methods. Viral cytoplasmic antigens were detected with MAb43.2, and both viral cytoplasmic and membrane antigens were detected with MAb79.0. Magnification, ×250.

Cells were either harvested or washed three times with serum-free medium, and the label was chased in normal medium for 10, 20, 30, 60, or 120 min in the absence or presence of tunicamycin (15 μg/ml). Cells were then washed three times with cold phosphate-buffered saline and disrupted in 4 ml of lysis buffer (0.02 M sodium phosphate [pH 7.6], 0.1 M NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS). Lysates were kept on ice for 2 h and centrifuged at 40,000 rpm in a Beckman SW60 rotor for 2 h at 5°C. Supernatants were stored at −70°C until immunoprecipitation was done.

Immunoprecipitation. In vitro translation products, suspended in 500 μl of lysis buffer, and VZV-infected cell lysates (700 μl) were incubated for 2 h at 4°C with 40 μl of a 10% Formalin-fixed suspension of protein A-containing *Staphylococcus aureus* Cowan I (21). After centrifugation at 9,000 × *g* for 20 min, VZV-specific proteins were immunoprecipitated at 4°C for 20 h in the presence of 50 μl of polyclonal or 100 μl of monoclonal antibodies prepared against VZV proteins. Finally, 30 μl of a 10% Formalin-fixed *S. aureus* suspension was added, and after 2 h at 4°C, absorbed immune complexes were washed three times with lysis buffer and suspended in 20 μl of TNE buffer (50 mM Tris [pH 7.4], 150 mM NaCl, 5 mM EDTA). After addition of 10 μl of 3× sample buffer (150 mM Tris [pH 7.0], 6% SDS, 15% 2-mercaptoethanol, 0.03% bromophenol blue), the suspension was heated in boiling water for 4 min, cooled on ice, and analyzed by 8 to 10% polyacrylamide SDS-PAGE (22, 37).

RESULTS

Reactivity of MAbs with translation products encoded by gpI and gpIV genes. Two MAbs (MAb43.2 and MAb79.0) were prepared against purified VZV virions. Indirect immunofluorescence assays of unfixed and acetone-fixed VZV-infected cells showed that MAb43.2 (IgG2) reacted only with cytoplasmic antigens of VZV-infected cells, whereas MAb79.0 (IgG2) recognized both cytoplasmic and membrane antigens in the infected cells (Fig. 2).

To test the reactivity of these MAbs with the primary translation products of gpI and gpIV, the predicted ORFs (4, 7) of VZV genes 67 (gpIV) and 68 (gpI) were subcloned from the *Sal*I-I DNA fragment (Fig. 1) into an in vitro transcription vector (pGEM) as described in Materials and Methods. The RNA transcribed from these genes was translated in vitro, and the IVT products were immunoprecipitated with MAbs and analyzed by SDS-PAGE. Figure 3A shows the primary translation products encoded by VZV genes 68 (70 kDa) and 67 (36 kDa). Immunoprecipitation of the IVT products with MAb79.0 revealed the 70-kDa and 36-kDa species, whereas MAb43.2 detected only the 36-kDa protein (Fig. 3B and C). These results indicated that MAb79.0 recognized epitopes on both gpI and gpIV and that MAb43.2 and MAb79.0 recognized different epitopes on gpIV. Additional protein bands smaller than 70 and 36 kDa were also detected by SDS-PAGE analysis and after immunoprecipitation of the IVT products. These proteins could be the

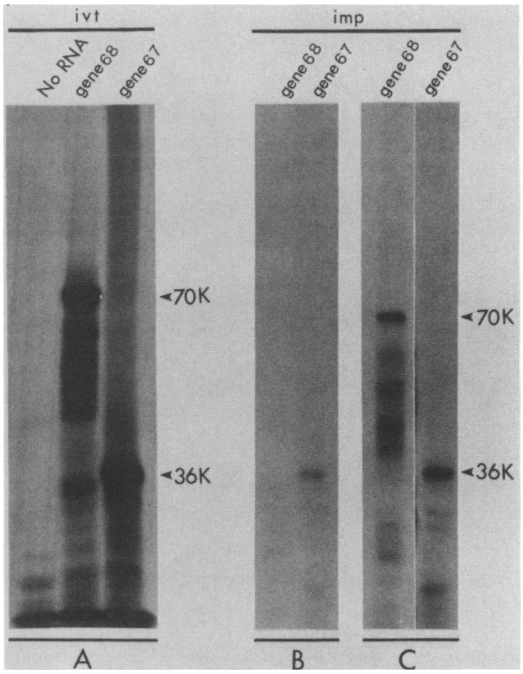

FIG. 3. In vitro translation (ivt) and immunoprecipitation (imp) of proteins encoded by VZV gpIV (gene 67) and gpI (gene 68). (A) VZV genes 67 and 68 were cloned into an in vitro transcription vector (pGEM), and RNA transcribed from these genes was translated in vitro. The IVT products were also immunoprecipitated with MAb43.2 (B) and MAb79.0 (C) and analyzed by SDS–8% polyacrylamide PAGE as described in Materials and Methods. As a control, RNA was excluded from the translation reaction (No RNA). Apparent sizes of the primary translation products (in kilodaltons) encoded by genes 67 and 68 are shown.

translation products of the truncated RNA species generated during the in vitro transcription of genes 67 and 68.

Analysis of VZV gpI and gpIV in the infected cells with MAbs. To identify the viral proteins recognized by MAb79.0 and MAb43.2, VZV-infected cells were pulse-labeled for 10 min and the label was chased for 120 min. Cell lysates were prepared and immunoprecipitated with MAbs as described in Materials and Methods. SDS-PAGE analysis of the immunoprecipitates showed that MAb79.0 precipitated an 82-kDa protein during a 10-min pulse which was processed during the chase period to the mature form (95 kDa) of VZV gpI (Fig. 4A) containing both N-linked and O-linked oligosaccharides (30). In the presence of tunicamycin, which inhibits the addition of N-linked oligosaccharides to the native protein, a smaller protein band of approximately 78 kDa was detected during the pulse period, which was processed to a 90-kDa protein containing only O-linked glycans (30). In addition, a 50-kDa protein was detected during a 10-min pulse. A 60-kDa protein, which is the processed form of gpIV (31, 33), was detected during the chase period. In the presence of tunicamycin, a 38-kDa protein was detected which was processed to 45 kDa, suggesting that gpIV may contain O-linked glycans and that the 45-kDa protein is the O-linked processed form of gpIV. After prolonged exposure of the gels (data not shown), a faint 36-kDa protein band was detected in the presence of tunicamycin, suggesting that MAb79.0 may also recognize the primary translation product of gpIV. Furthermore, the formation of 50-kDa and 60-kDa proteins was inhibited by tunicamycin. This observation was consistent with the previous findings (31) that the 50-kDa (high-mannose intermediate) protein is processed to the 60-kDa protein containing N-linked oligosaccharides.

Pulse-chase analysis of VZV-infected cells with MAb43.2 revealed a major 36-kDa protein during the pulse, which was processed to a 38-kDa and a 45-kDa protein during the chase (Fig. 4B). These results were consistent with those obtained with MAb79.0; however, MAb43.2 did not precipitate the high-mannose intermediate (50 kDa) or processed form (60 kDa) of gpIV. In addition, MAb43.2 precipitated a faint protein band of approximately 105 kDa. Similar results have been found when VZV-infected cells were immunoprecipitated with monoclonal and rabbit antipeptide antibodies produced against gpIV (8, 31). Whether the 105-kDa protein is related to gpI or is a distinct viral protein that coprecipitates with MAb43.2 remains to be determined.

Together, the results of pulse-chase experiments followed by immunoprecipitation with MAbs indicated that (i) MAb79.0 reacted with precursor products of gpI (78, 82, 90, and 95 kDa) and pgIV (38, 45, 50, and 60 kDa) and (ii) MAb43.2 recognized only the precursor protein (36 kDa) and O-linked forms (38 and 45 kDa) of gpIV. Immunoprecipitation of [^{3}H]mannose-labeled infected-cell lysates with MAbs followed by SDS-PAGE analysis showed that MAb79.0 precipitated the high-mannose intermediates (50 and 82 kDa) and the processed forms of gpIV (60 kDa) and gpI (95 kDa), whereas MAb43.2 did not react with these proteins (Fig. 5A). Similar results were also obtained when [^{3}H]fucose-labeled VZV-infected cell lysates were immunoprecipitated with MAb43.2 (data not shown).

Analysis of VZV gpI and gpIV with polyclonal antibodies. VZV-infected cells were also immunoprecipitated with polyclonal antibodies which recognize the precursor products of both gpI and gpIV. Polyclonal antibodies were produced in rabbits (RAnti-gpI and -IV) against the primary translation products of gpIV and gpI, encoded by VZV genes 67 and 68, respectively. VZV-infected cells were pulse-labeled for 10 min, and the label was chased for 10, 20, 30, and 60 min. Cell lysates were immunoprecipitated with RAnti-gpI and -IV and analyzed by SDS-PAGE. Three protein bands of 36, 50, and 82 kDa were detected during a 10-min pulse which were processed to the mature forms of gpIV (60 kDa) and gpI (95 kDa) during a 60-min chase period (Fig. 4C). In the presence of tunicamycin, a 36-kDa and a 78-kDa protein were observed during the pulse period which were processed to a 38-, a 45-, a 78-, and a 90-kDa form during the chase period (Fig. 4D). These results were consistent with those obtained with MAb79.0 and 43.2 and indicated that RAnti-gpI and -IV recognized both the precursor and mature forms of gpI and gpIV. In addition, recognition of only two primary translation products by RAnti-gpI and -IV is in agreement with the predicted genetic expression of the VZV genome (7) and indicate that no other ORF exists within the coding regions of VZV genes 67 (gpIV) and 68 (gpI).

Phosphorylation of VZV gpIV. To determine whether gpIV is phosphorylated, VZV-infected cells were labeled with $^{32}P_i$, and cell lysates were precipitated with MAb43.2 and with rabbit anti-VZV antibodies (RAnti-VZV) prepared against purified virions (36). Three phosphorylated proteins (36, 38, and 45 kDa) were precipitated with MAb43.2, whereas RAnti-VZV antisera detected these phosphoproteins in addition to VZV gpI (Fig. 5B).

Identification of amino acid residues on gpI comprising the MAb-binding site. To map the binding site (epitope) of MAb79.0, two truncated DNA fragments were first generated by digestion of the gpI gene with *Cla*I and *Bgl*II

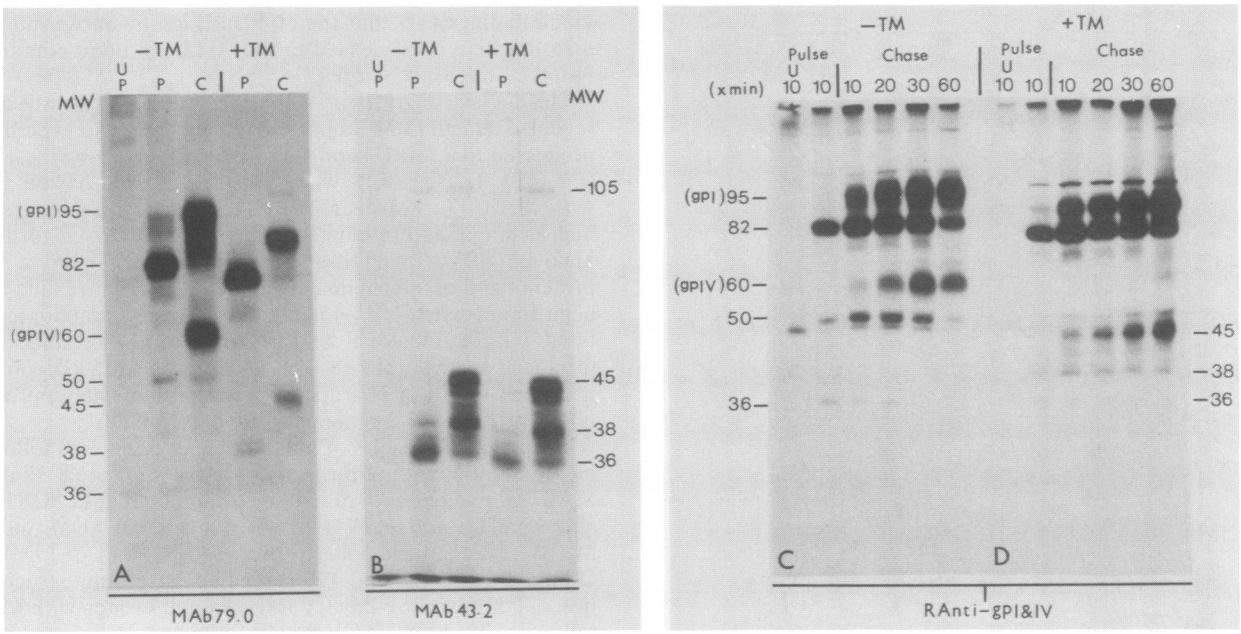

FIG. 4. (A and B) Pulse-chase experiments and immunoprecipitation of VZV-infected cells with MAbs. Cells (10^5) were infected with VZV, and at 48 h postinfection, the cells were pulse-labeled (lanes P) with [^{35}S]methionine (300 μCi/ml) for 10 min in the absence (−) or presence (+) of tunicamycin (TM). Cells were either harvested or washed, and the label was chased (lanes C) for 120 min. Cell lysates were prepared, immunoprecipitated with MAb79.0 (A) or MAb43.2 (B), and analyzed by SDS–8% polyacrylamide PAGE as described in Materials and Methods. Lanes U, Uninfected cells were pulse-labeled for 10 min. (C and D) Immunoprecipitation of VZV-infected cells with polyclonal antibodies directed against VZV gpIV and gpI. Infected cells were pulse-labeled with [^{35}S]methionine (300 μCi/ml) for 10 min, and the label was chased for 10, 20, 30, or 60 min in the presence or absence of tunicamycin. Cell lysates were immunoprecipitated with antibodies raised in a rabbit (RAnti-gpI and -IV) against the IVT products encoded by VZV gpIV (gene 67) and gpI (gene 68) and analyzed by SDS–10% polyacrylamide PAGE. Lanes U, Uninfected cells were pulse-labeled for 10 min. Apparent sizes (in kilodaltons) of the precursor products of VZV gpIV and gpI recognized by MAbs and RAnti-grpI and -IV are shown. Lysozyme (14.3 kDa), β-lactoglobulin (18.4 kDa), α-chymotrypsinogen (25.7 kDa), ovalbumin (43.0 kDa), bovine serum albumin (68.0 kDa), phosphorylase B (97.4 kDa), and myosin (200.0 kDa) were used as internal size markers.

endonucleases downstream from the SP6 promoter at 109 and 160 residues of the predicted amino acid sequences of gpI (7), respectively. RNA was transcribed from the full-length and truncated gpI DNA fragments and translated in vitro, and the IVT products were immunoprecipitated with MAb79.0 and human sera (H-sera). Figure 6A shows immunoprecipitation of IVT products encoded by the entire coding region of gpI. Immunoprecipitation of IVT products encoded by the *Bgl*II-digested gpI gene with both MAb79.0 and H-sera resulted in the detection of an 18-kDa protein (Fig. 6B, lanes 2 and 5), whereas no immunoprecipitation was detected when the IVT products encoded by the *Cla*I-digested gpI gene were reacted with these antibodies (Fig. 6B, lanes 3 and 6). These results indicated that the binding site of MAb79.0, which is also recognized by H-sera, is located within 51 amino acid residues between the *Cla*I and *Bgl*II sites. To further localize the MAb79.0 epitope within the 51 amino acid residues, the gpI gene was digested with *Xho*II downstream from the *Cla*I site at residue 123 of gpI. When the IVT products, encoded by RNA transcribed from this truncated DNA, were immunoprecipitated with MAb79.0 and H-sera, a 16-kDa protein band was detected by both antibodies (Fig. 6C, lanes 2 and 4), indicating that the Mab79.0 binding site is located within the 14 amino acid residues (residues 109 to 123 on gpI) between the *Cla*I and *Xho*II sites. By comparison of the predicted amino acid sequences of VZV gpI and gpIV (7), we found that 14 amino acid residues on gpI (residues 107 to 121) had a degree of similarity (36%) to two regions (residues 55 to 69 and 245 to 259) of gpIV (Fig. 7), suggesting that MAb79.0 may recognize similar epitopes on both VZV gpI and gpIV.

DISCUSSION

The results presented in this study provide evidence for recognition of VZV gpI and gpIV with one MAb. This distinction was determined in part by the recognition of the IVT products encoded by gpI and gpIV genes with this MAb (MAb 79.0). In addition, immunoprecipitation studies of VZV-infected cells with MAb79.0 and RAnti-gpI and -IV indicated that this MAb recognized the precursor and mature forms of both gpI and gpIV. Furthermore, our results indicated that MAb79.0 binds to an antigenic site located within 14 amino acid residues (residues 109 to 123) on gpI. From the predicted amino acid sequences, we found that 14 amino acid residues on gpI (residues 107 to 121) display a degree (36%) of sequence similarity to two regions on gpIV (residues 55 to 69 and 245 to 259). These findings suggest that the reactivity of MAb79.0 with different gene products could be due to the existence of similar antibody-binding sites on gpI and gpIV which are recognized by one MAb. Reactivity of a single MAb with a VZV glycoprotein complex (gp98-gp62) has been reported (30). Since the sizes of gp98 and gp62 correspond to those of VZV gpI and gpIV, respectively, it might be expected that, like MAb79.0, the MAb used by Montalvo et al. (30) recognizes similar epitopes on both gpI and gpIV.

It has been shown that HSV gE, which contains Fc-binding activity (2), cannot bind IgG and that HSV gI and gE

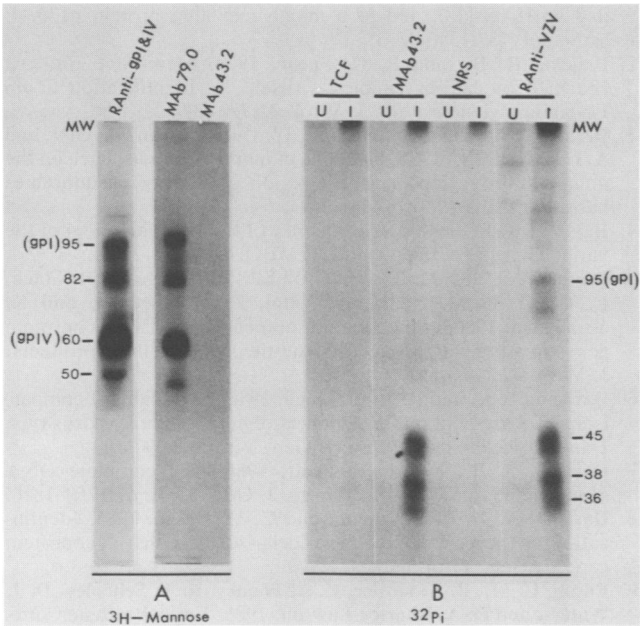

FIG. 5. (A) Immunoprecipitation of [^{3}H]mannose-labeled VZV-infected cells. Cells were infected with VZV, and at 48 h postinfection, cells were labeled with [^{3}H]mannose (500 μCi/ml) for 4 h. Cell lysates were prepared, immunoprecipitated with rabbit anti-gpI and -IV, MAb 79.0, and MAb 43.2 and analyzed by SDS–8% polyacrylamide PAGE. Apparent sizes (in kilodaltons) of high-mannose intermediates and mature forms of VZV gpIV and gpI are shown on the left. (B) Immunoprecipitation of ^{32}P-labeled VZV-infected cells. Uninfected (lanes U) and VZV-infected (lanes I) cells (10^5) were labeled with $^{32}P_i$ (500 μCi/ml) for 2 h. Cell lysates were prepared, immunoprecipitated with tissue culture fluid (TCF) of SP2/O mouse myeloma cells, MAb43.2, normal rabbit serum (NRS), and rabbit anti-VZV antibodies (RAnti-VZV). VZV gpI and apparent sizes (in kilodaltons) of proteins recognized by MAb43.2 and RAnti-VZV are shown on the right.

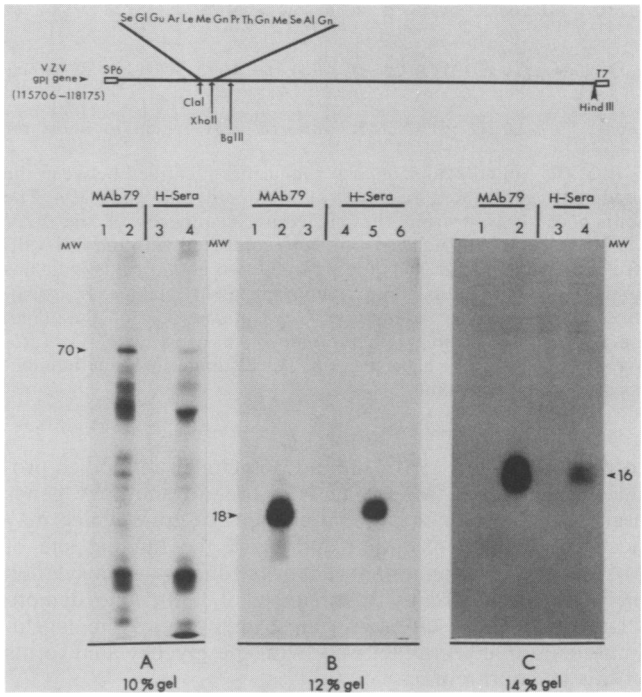

FIG 6. Identification of an antibody-binding site (epitope) on the primary translation product of VZV gene 68 (gpI). The VZV *Bgl*I DNA fragment (2,469 bp), which is located between nucleotides 115,706 and 118,175 of the VZV genome (7) and contains the coding region of gpI gene, was cloned into the pGEM transcription vector downstream from the SP6 promoter. The recombinant plasmid was linearized with *Hin*dIII downstream from the *Bgl*I DNA insert, and three DNA fragments were generated by digestion of the DNA insert with *Cla*I, *Xho*II, and *Bgl*II endonucleases downstream from the SP6 promoter, as described in Materials and Methods. RNA was transcribed from the entire coding region of gpI and from each truncated gpI DNA fragment. These RNA transcripts were translated in vitro in the presence of [^{35}S]methionine in a rabbit reticulocyte lysate as described (35). The translation products were immunoprecipitated with MAb79 and H-sera and analyzed by 10 to 14% polyacrylamide SDS-PAGE as described in Materials and Methods. (A) Immunoprecipitation of the IVT products encoded by the entire coding region of gpI (lanes 2 and 4). In lanes 1 and 3, no RNA was included in translation reactions. The arrow indicates the gpI primary translation product with an apparent size of 70 kDa which was encoded by a full-length RNA transcript. (B) Immunoprecipitation of the translation products (18 kDa) encoded by the gpI gene digested with *Bgl*II (lanes 2 and 5) and *Cla*I (lanes 3 and 6). In lanes 1 and 4, no RNA was included in the translation reactions. (C) Immunoprecipitation of the translation products (16 kDa) encoded by the gpI gene digested with *Xho*II (lanes 2 and 4). In lanes 1 and 3, RNA was excluded from the translation reactions. The gpI epitope recognized by MAb79.0 and H-sera is located within 14 amino acid residues (Ser-Gly-Glu-Arg-Leu-Met-Gln-Pro-Thr-Gln-Met-Ser-Ala-Gln) between the *Cla*I and *Xho*II sites.

form a complex which binds IgG (18). Therefore, the association of gI and gE appears to be important in recognition of IgG. It is not known whether VZV gpI and gpIV form a complex similar to that of HSV gI and gE. Since no Fc-binding activity has been detected for VZV gpI or other VZV glycoproteins (10), the association of gpI and gpIV, if any, may involve different mechanisms.

Our studies with MAb43.2, which reacts with the primary translation products of gene 67, identified three phosphorylated precursor species to gpIV. In immunoprecipitation analysis of VZV-infected cells, MAb43.2 precipitated a 36-kDa phosphoprotein which was processed to a 38-kDa and a 45-kDa phosphoprotein. Thus, the 36-kDa form appears to be the precursor of the 45-kDa protein, which may contain O-linked oligosaccharides, and which is in turn processed to a high-mannose intermediate (50 kDa) and finally to the mature form (60 kDa) of gpIV by addition of N-linked glycans (31).

The results indicated that MAb43.2 reacted only with cytoplasmic antigens of VZV-infected cells and did not precipitate the mature form of gpIV, which is present on the membrane of the infected cells (31). In contrast, MAb79.0 reacted with both cytoplasmic and membrane antigens of the infected cells and precipitated the precursor and processed forms of gpI and gpIV, suggesting that glycosylation of gpIV ablates its recognition by MAb43.2. In addition, MAb43.2 reacted with the translation products of the gpIV gene but not that of gpI, suggesting that MAb79.0 and MAb43.2 recognize different epitopes on gpIV. It is therefore possible that during the process of glycosylation, the binding site of MAb43.2 is masked and thus becomes unavailable for recognition by this MAb. Another possibility is that gpI and gpIV form a complex on the cell surface which masks the MAb43.2 binding site. The possibility also exists that a different pathway may be involved, in which the phosphorylated form (45 kDa) of gpIV may be a terminal species which never becomes glycosylated to form the 60-kDa species. However, recognition of the precursor and mature

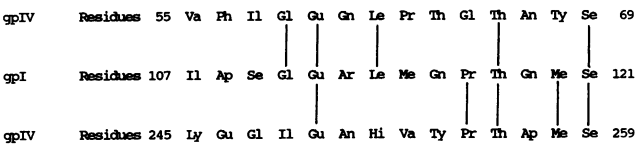

FIG. 7. Amino acid sequence similarities identified between the predicted amino acid residues of VZV gpI and gpIV (7). The sequences derived from the nucleotide sequences of the VZV genome (a gift from A. Davison) were compared with the DNASIS program (Hitachi, Inc.). A line between two amino acids indicates similarity. Abbreviations: Va, valine; Ph, phenylalanine; Il, isoleucine; Gl, glycine; Gu, glutamine; Gn, glutamic acid or glutamine; Le, leucine; Pr, proline; Th, threonine; An, asparagine; Ty, tyrosine; Se, serine; Ap, aspartic acid; Ar, arginine; Me, methionine; Ly, lysine; Hi, histidine.

forms of gpIV by MAb79.0 and polyclonal antibodies produced against the IVT products of the gpI and gpIV genes argues for the conformational changes of gpIV which may occur during glycosylation and mask the binding site of MAb43.2. Conformational changes during glycosylation have been reported by Waxham et al. (38), who demonstrated that MAbs directed against mumps virus hemagglutinin-neuraminidase react only with the glycosylated forms of this glycoprotein.

Previous studies have shown that the high-mannose intermediate and mature forms of VZV gpI are phosphorylated (29, 36). The results presented herein demonstrated that the precursor proteins but not the final product of VZV gpIV are phosphorylated, as detected by MAb43.2 and rabbit anti-VZV polyclonal antibodies. Therefore, it appears that the primary translation product of gpIV becomes phosphorylated. However, the phosphate moieties may be removed during the final stages of gpIV glycosylation. On the other hand, it is the high-mannose and the mature products of gpI which are phosphorylated and not the primary translatin product of gene 68. VZV gpI has been shown to become phosphorylated with a VZV-infected protein kinase (29) which may be encoded by VZV gene 66 (7), located upstream from VZV gene 67 (gpIV), and therefore may contribute to the phosphorylation of both gpIV and gpI. The biological function of phosphorylation and dephosphorylation of VZV glycoproteins is not known. However, selective phosphorylation of VZV gpI and gpIV and HSV gE (10) may contribute to similar functions shared among alphaherpesviruses. Since VZV gpI and HSV gE have been shown to be linked to fatty acids (10, 19), it is possible that these molecules have biological functions similar to those of membrane-anchored phosphoglycoproteins in playing a role in translocation and release of viral glycoproteins across the cell surface membrane (25).

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