Antibody Response to Varicella-Zoster Virus Surface Glycoproteins in Chickenpox and Shingles

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SUMMARY

Varicella-zoster virus (VZV)-infected cell surface proteins were investigated using extrinsic radiolabelling of the cell surface, immunoprecipitation of detergent-solubilized extract of the same cell surface and fractionation of the immunoprecipitates using SDS–PAGE. Glycosylated proteins were identified by their affinity for *Ricinus communis* lectin. Six glycoproteins with apparent mol. wt. of 170K, 105K, 93K, 81K, 53K and 45K were identified. The 170K glycoprotein was shown to be disulphide-linked since under reducing conditions for SDS–PAGE it was cleaved to a protein of 63K mol. wt. The IgG responses to these glycoproteins during various clinical circumstances are described. In acute sera from all chickenpox patients and in the majority of acute shingles sera, antibodies reactive with glycoproteins could not be detected. In chickenpox convalescence, antibodies reactive with glycoproteins of mol. wt. 170K, 105K, 53K and 45K were identified, whilst during zoster convalescence antibodies to all six were prominent. Antibodies to the disulphide-linked glycoprotein persisted for many years following both the primary disease and its reactivation. Disseminated zoster was associated with significantly low levels of antibodies to these surface glycoproteins.

INTRODUCTION

Primary infection with varicella-zoster virus (VZV) causes chickenpox (varicella) whilst subsequent reactivation of latent virus can result in the secondary manifestation shingles (zoster). Association of severe infection with immunodeficiency disease has been reported for varicella (Feldman *et al*., 1975) while zoster increases in severity and frequency of reactivation in immunocompromised patients (Goffinet *et al*., 1972). Shore & Feorino (1981) suggested that cellular immunity was more important than humoral immunity in VZV infections. However, zoster immunoglobulin (ZIG) may prevent or modify VZV infection (Brunell & Gershon, 1973). Further, high titre ZIG can modify varicella in the immunocompromised person (Brunell *et al*., 1969).

Investigation of VZV proteins has been carried out in various laboratories using different systems of virus propagation and purification. The virus proteins studied were intrinsically labelled with $^{35}$S)methionine, $^{14}$C)glucosamine or $^{3}$H)fucose and characterized by immunoprecipitation. Asano & Takahashi (1980) described 31 polypeptides of which seven were glycosylated. The glycoproteins had molecular weights (mol. wt.) of $105 \times 10^3$, $90 \times 10^3$, $84 \times 10^3$, $72 \times 10^3$, $70 \times 10^3$, $60 \times 10^3$ and $56 \times 10^3$ (105K, 90K, 84K, 72K, 70K, 60K and 56K). Grose (1980) identified five glycoproteins with mol. wt. of 118K, 98K, 88K, 62K and 45K designated gp118, gp98, gp88, gp62 and gp45 respectively. Zweerink & Neff (1981) detected three glycoproteins with mol. wt. of 130K, 88K and 60K. Grose *et al.* (1984) later resolved a 140K disulphide-linked VZV glycoprotein, gp140. Identification of gp140, gp98, and gp62 in association with the infected cell membrane was confirmed using monoclonal antibodies in both viable and fixed cell immunofluorescence tests (Weigle & Grose, 1983; Grose *et al*., 1984).
Antibody responses to viral antigens following VZV infection have been reported for few patients. Zweerink & Neff (1981) reported immunoprecipitation studies on nine patients; antibodies to 14 polypeptides including three glycosylated ones were described. Grose (1983) detected antibodies to 16 polypeptides, of which three were glycosylated, in sera from five children with zoster. Weigle & Grose (1984) studying sequential sera from 10 patients with VZV infection determined antibodies to four glycoproteins, gp118, gp98, gp66 and gp62 in sera from patients convalescing from chickenpox. Only antibodies to gp118 and gp66 were detected 4 years later. In convalescent zoster sera the antibody responses to gp98 and gp62 were greater than after chickenpox and antibodies to three further glycoproteins gp107, gp88 and gp45 were present. Antibodies to gp118 and gp66 persisted long after zoster.

In the present study, we have characterized the polypeptides expressed on the surface of VZV-infected cells, identified the immunogenic glycopeptides and determined the humoral immune response to those glycopeptides in different clinical circumstances using extrinsically labelled virus-specific surface proteins. The details of the primary and secondary antibody responses are documented.

METHODS

Cells and virus. Cells from the Wong-Kilbourne derivative (D) of Chang conjunctival clone 1-5c-4 (Flow Laboratories) were grown in Eagle's TC199 medium (Wellcome) supplemented with 10% foetal calf serum and 2 g/l sodium bicarbonate (GM); cells were maintained on the same medium containing only 2% foetal calf serum (MM). Mycoplasma-free cells were used; frequent checks for contamination were performed with Hoechst 33258 reagent (Russell et al., 1975).

The VZV used was isolated from an infant with varicella. Following primary isolation in MRC-5 tissue culture cells, virus was grown in the Chang conjunctival cells and used between passages 5 and 15.

Sera and patients. Acute sera from five patients and early convalescent sera from six, collected 14 to 24 days after onset of varicella, and 20 sera collected from adults aged 24 to 88 years known to have had childhood varicella and no past history of zoster, were studied.

Acute zoster sera from six adults and post-zoster sera from 15 adults, 10 collected in early convalescence (3 to 9 days after onset) and five collected 3 to 15 years after the disease, were also studied.

In addition, early convalescent zoster sera collected from four adults on the 4th to 10th day of severe disease, demonstrated by extensive vesiculation involving more than one dermatome, were examined. One patient experienced gastric zoster, and all were sufficiently ill to be hospitalized.

Preparation of $^{125}$I-labelled antigen. A subconfluent monolayer (75 cm$^2$) of Chang cells was inoculated with 1 x 10$^7$ VZV-infected Chang cells in 3 ml GM. After 1.5 h adsorption, 20 ml GM was added and cells were reincubated at 37°C for 5 h. The supernatant solution was decanted and unattached cells washed off before removing the remaining adherent cells with 0.1% EDTA and 0.05% trypsin (Wellcome). Resuspended cells were seeded in 50 ml GM into a sterile 13 cm diam. dish. The cells were removed by gentle pipetting, centrifuged at 600 g for 5 min, washed in phosphate-buffered saline (PBS, Oxoid) containing 1 mM-phenylmethylsulphonyl fluoride (PMSF), twice in PBS, then resuspended in 1 ml PBS (2 x 10$^7$ cells).

The cell suspension was placed in a glass bijou and 2 mCi Na$^{125}$I (15 mCi/μg; Amersham) were added followed by two iododebuses (Pierce, Rockford, Ill., U.S.A.). The reaction was allowed to proceed with occasional shaking at room temperature for 5 min and was stopped by washing the cells in serum-free TC199 containing 2.5 mM-cysteine. Labelled cells were washed three times in PBS containing 1 mM-PMSF.

A soluble cell extract was prepared. The cells were incubated at 37°C for 1 h, then at 4°C overnight in 1 ml 10 mM-Tris-HCl pH 7.4 containing 0.1 mM-EDTA, 1 mM-PMSF, 0.5% (v/v) NP40 (Sigma) and 0.5% (w/v) sodium deoxycholate. Cells were removed by centrifugation at 12000 g for 5 min and the supernatant solution was centrifuged at 100000 g for 20 min to remove insoluble aggregates. The supernatant containing $^{125}$I-labelled proteins was stored at -20°C. Mock-infected cells were similarly iodinated.

Radioimmunoprecipitation assay (RIPA). A sample of solubilized $^{125}$I-labelled proteins (5 x 10$^5$ c.p.m.) was incubated with 10 μl serum, and Protein A-Sepharose beads [Sigma, 50 μl of a 10% (w/v) suspension in PBS] in a total volume of 250 μl RIPA buffer: 10 mM-Tris-HCl pH 7.4, 0.15 M-NaCl, 0.5% (w/v) Empigen BB (Albright & Wilson Ltd., Whitehaven, U.K.), 0.1% (w/v) SDS and 100 μM-PMSF. The reactants were mixed for 4 h at 4°C. The beads were sedimented by centrifuging at 12000 g for 1 min and unbound reactants discarded. The beads were washed extensively in RIPA buffer and finally in PBS before eluting the immune complexes at 100°C into dissociation buffer consisting of 0.25 M-Tris-HCl pH 6.8, 4% SDS, 20% (v/v) glycerol, 10% (v/v) 2-mercaptoethanol, 0.002% bromophenol blue, and centrifuging at 12000 g for 1 min. The eluates were analysed by SDS-PAGE.

For identification of disulphide-linked proteins immune complexes were eluted into dissociation buffer without 2-mercaptoethanol.
Identification of glycosylated polypeptides. Labelled proteins (1 × 10⁶ c.p.m.) were reacted overnight with gentle shaking at 4 °C with 100 µl *Ricinus communis* agglutinin I, bound to agarose beads (Vector Laboratories, Burlingame, Ca., U.S.A.) in 2 ml 10 mM-Tris–HCl pH 7.1 containing 0.5% (w/v) CHAPS (3-[3-(cholamidopropyl)-dimethylammonio] 1-propane sulphonate), 0.15 M-NaCl, 1 mM-MnCl₂ and 1 mM-CaCl₂ (TC buffer). Glycoproteins containing α- and β-D-galactose groups bind to *Ricinus communis* lectin. Unbound reactants were discarded following centrifugal sedimentation of the beads. The beads were washed three times and resuspended in 200 µl TC buffer before reacting overnight at 4 °C with 10 µl convalescent zoster serum. Beads were washed three times before eluting bound immune complexes with 1 ml 100 mM-lactose pH 8. The eluate was reacted with Protein A-Sepharose for 4 h at 4 °C and washed as in the RIPA before dissociating and analysing by SDS-PAGE and autoradiography.

**SDS–PAGE and autoradiography.** Polyacrylamide slab gels containing 0-1% SDS were prepared from acrylamide cross-linked with N,N'-methylenebisacrylamide (MBA) constituting 2.5% (w/w) of the total acrylamide. Electrophoresis of solubilized samples under reducing or non-reducing conditions was at 200 V for 20 h at 4 °C using the discontinuous buffer system of Laemmli (1970) with a linear 10 to 25% (w/v) acrylamide gradient as described by Heckels (1981). Dried gels were exposed to Kodak XAR-5 X-ray-sensitive film in a radiographic cassette fitted with a Dupont Lightning intensifying screen at -70 °C for 3 to 9 days. Molecular weight marker proteins (Sigma) were myosin (205K), β-galactosidase (116K), phosphorylase b (98K), catalase (58K), actin (42-3K), trypsinogen (24K) and myoglobin (17-2K).

**Quantification of antibody response.** For quantification of total IgG antibody response undiluted sera were reacted with labelled proteins using the RIPA method described above, the total c.p.m. precipitated were recorded and the means determined for acute and convalescent sera from both varicella and zoster patients. The quantification of the antibody response to specific proteins was performed following SDS-PAGE analysis of the immune precipitates prepared by the RIPA method. Sera taken from zoster patients in early convalescence were diluted 1:16 in 0.5~ bovine serum albumin (BSA) in PBS; all other sera were not diluted. Bands were located by autoradiography, excised and radioactivity of each immunoprecipitate measured in an LKB 12802; counter. The counts were corrected for background by subtracting the mean count of five bands excised from gel lanes of antigen immunoprecipitated with pre-varicella serum.

**Analysis of results.** Student's t-test was used to compare means of the immunoprecipitate counts.

**Fluorescent antibody test (FAT) procedure for detecting antibodies to VZV in human sera.** Sera were titrated on slides containing fixed VZV-infected cell monolayers as described previously (Larkin *et al.*, 1983). Fluorescence was assessed on a scale + + + + to + according to intensity. The titre was recorded as the reciprocal of the lowest dilution giving a + fluorescence.

**RESULTS**

**Identification of VZV surface antigens**

VZV surface proteins were identified following immunoprecipitation of lysates of ¹²⁵I-labelled infected Chang conjunctival cell surface. Using a high titre convalescent zoster serum (FAT 1:4096) VZV antigenic proteins were identified by RIPA followed by SDS-PAGE and autoradiography (Fig. 1a). Under reducing conditions six proteins with mol. wt. of 105000, 93000, 81000, 63000, 53000 and 45000 were identified. All six were shown to be glycosylated by their affinity for *Ricinus communis* lectin and subsequent immunoprecipitation with the same convalescent zoster serum (Fig. 1b).

In contrast, under non-reducing conditions the 63K glycoprotein was absent from the electrophoretic profile (Fig. 2). Accompanying the loss of this glycoprotein was the presence of a previously undetected protein of apparent mol. wt. 170000 which was shown to be glycosylated by its affinity for *Ricinus communis* lectin. The five proteins of mol. wt. 105K, 93K, 81K, 53K and 45K appeared unaltered under non-reducing conditions.

All defined proteins were immunoprecipitated from VZV-infected cells only when using VZV immune sera. Where uninfected cells were reacted with immune serum or where VZV-infected cells were reacted with acute varicella sera no bands were detected. Neither were they detected when acute varicella serum with high titre antibody to herpes simplex virus was reacted with VZV-infected cells.

**Quantification of antibody response to total immunoprecipitated antigen**

Acute and early convalescent varicella and zoster sera were reacted with labelled antigen using the RIPA method. Convalescent zoster sera precipitated significantly greater c.p.m. (P < 0.01) of total lysate than did varicella sera (Fig. 3).
Fig. 1. RIPA and SDS-PAGE analysis on a 10 to 25% (w/v) acrylamide gradient. (a) $^{125}$I-labelled VZV-infected Chang conjunctival cell surface proteins immunoprecipitated with convalescent zoster serum (FAT titre = 4096); (b) Ricinus communis-bound $^{125}$I-labelled VZV-infected Chang conjunctival cell surface glycoproteins immunoprecipitated with convalescent zoster serum (FAT titre = 4096). The gel was exposed to film for 4 days.

Fig. 2. RIPA and SDS-PAGE analysis on a 10 to 25% (w/v) acrylamide gradient, under (a) non-reducing conditions and (b) reducing conditions of $^{125}$I-labelled VZV-infected Chang conjunctival cell surface proteins immunoprecipitated with convalescent zoster sera. Lanes 1 and 4, serum 1, FAT titre = 4096; lanes 2 and 5, serum 2, FAT titre = 2048; lanes 3 and 6, serum 3, FAT titre = 2048. The gel was exposed to film for 4 days.
Antibodies to VZV surface glycoproteins

Characterization of antibody response following varicella

Acute and early convalescent varicella sera from six patients were reacted with $^{125}$I-labelled proteins in a RIPA and analysed by SDS–PAGE. No antibodies could be detected in any of the acute sera. However, all varicella convalescent sera contained antibodies to at least four viral polypeptides, the 170K glycoprotein resolved as a 63K subunit in reduced gels [170(63)K] and the glycoproteins of 105K, 53K and 45K mol. wt. A less pronounced antibody response was noted to the 93K and 81K glycoproteins (Fig. 4a). Distortion due to IgG heavy chain caused compression of the 53K band.

In sera from 14 adults between 25 and 74 years of age with known childhood varicella, antibodies to VZV glycoprotein 170(63)K were detected. Antibodies to 105K were present in sera from nine of these adults whilst in 12 sera antibodies to the 53K and 45K glycoproteins were also detected. Fig. 4(b) demonstrates the electrophoretic profile of sera from three of these adults. In sera from six patients $\geq 59$ years of age VZV antibodies to all surface proteins were undetectable under the conditions used.

Characterization of antibody response following zoster

Acute and convalescent zoster sera were reacted with labelled proteins in a RIPA and analysed by SDS–PAGE. Low levels of antibodies to the 170(63)K, 105K, 93K and 53K glycoproteins could be detected in one of six acute sera tested (FAT titres 32, 32, 32, 16, 16, 8). However, all convalescent sera collected 3 to 9 days after onset contained antibodies to six viral polypeptides of mol. wt. 170(63)K, 105K, 93K, 81K, 53K and 45K (Fig. 5).

In sera collected 3 to 15 years following zoster from five adults, four contained antibodies to the 170(63)K glycoprotein. Antibodies to 105K, 53K and 45K glycoproteins were also detected in three of the sera.
Fig. 4. RIPA and SDS-PAGE on a 10 to 25% (w/v) acrylamide gradient, of convalescent varicella sera assayed for their content of antibody to cell surface-associated VZV antigen. The antigen was $^{125}$I-labelled infected Chang conjunctival cell surface. (a) Six sera taken 2 to 3 weeks post-infection. Lane 1, serum 1, FAT titre = 128; lane 2, serum 2, FAT titre = 512; lane 3, serum 3, FAT titre = 1024; lane 4, serum 4, FAT titre = 1024; lane 5, serum 5, FAT titre = 2048; lane 6, serum 6, FAT titre = 4096. (b) Three sera taken 10 to 20 years post-infection. Lane 7, serum 7, FAT titre = 128; lane 8, serum 8, FAT titre = 128; lane 9, serum 9, FAT titre = 32. The gels were exposed to film for (a) 4 days and (b) 8 days.

Fig. 5. RIPA and SDS-PAGE on a 10 to 25% (w/v) acrylamide gradient, of convalescent zoster sera assayed for their content of antibody to cell surface-associated VZV antigen. The antigen was $^{125}$I-labelled infected Chang conjunctival cell surface. Sera were taken 3 to 11 days post-infection. Lane 1, serum 11, FAT titre = 2048; lane 2, serum 12, FAT titre = 512; lane 3, serum 13, FAT titre = 4096; lane 4, serum 14, FAT titre = 1028; lane 5, serum 15, FAT titre = 4096; lane 6, serum 16, FAT titre = 2048; lane 7, serum 17, FAT titre = 256; lane 8, serum 18, FAT titre = 2048; lane 9, serum 19, FAT titre = 2048. The gel was exposed to film for 3 days.
Antibodies to VZV surface glycoproteins

Fig. 6. Serum antibody level to (a) 105K, (b) 93K, (c) 81K, (d) 63K, (e) 53K and (f) 45K. Antigen bands from RIPA of 125I-labelled antigen were located by autoradiography, excised, the gel slices placed in vials and γ-counted. ●, Sera from 10 convalescent zoster patients (FAT reciprocal titres 2048, 2048, 2048, 2048, 4096, 4096, 512, 2048, 512, 2048, 4096); ○, sera from four patients with disseminated zoster (FAT reciprocal titres 64, 128, 64, 16). [All sera were diluted 1:16 in 0.5% (w/v) BSA in PBS.]

Quantification of antibodies to VZV surface antigens during disseminated zoster

Early convalescent sera from four patients with disseminated zoster were reacted with labelled proteins in a RIPA and analysed by SDS–PAGE and autoradiography. A significant reduction ($P < 0.01$) in immunoprecipitation of the major surface glycoproteins was noted when compared with sera from normal zoster (Fig. 6). The mean number of days from onset was 6 days for the disseminated zoster group whilst for the normal patients it was 6.5 days.

DISCUSSION

Virus-coded glycoproteins on the outer surface of the herpesvirus envelope are essential for viral infectivity and replication (Ghosh, 1980). During VZV replication insertion of virus-specific glycoproteins into the HeLa cell membrane is recognized as early as 12 h post-infection (Ogata & Shigeta, 1979).

Antibody binding to virus-coded surface proteins plays a significant role in recovery from infection (Sissons & Oldstone, 1980). Mechanisms include complement-enhanced cell killing via the classical and alternate pathways (Hicks et al., 1976). Specific mechanisms include inhibition of cell to cell spread of VZV (Shigeta & Ogata, 1979), cell killing via lymphoid cells (ADCC) (Gershon & Steinberg, 1981) and neutralization (Taylor-Robinson, 1959; Caunt & Shaw, 1969).

In the present study, using the RIPA technique the VZV-coded antigenic proteins on the infected cell surface were identified. Under reducing conditions in SDS–PAGE six viral glycoproteins were revealed in the electrophoretic profile with apparent mol. wt. of 105K, 93K, 81K, 63K, 53K and 45K. In the nomenclature of Grose (1980), Grose & Friedrichs (1982) and Grose et al. (1984) these proteins are likely to correspond to gp118, gp98, gp88, gp66, gp62 and gp45. In contrast, under non-reducing conditions an additional high molecular weight glycoprotein was identified, its apparent mol. wt. in this study being 170000. Accompanying the appearance of this 170K glycoprotein was the loss of the 63K band from the profile. These findings strongly indicate that the 170K glycoprotein is disulphide-linked, present on infected cell membranes and is immunogenic. Further, subsequent cleavage of the disulphide bonds of the 170K glycoprotein produces a subunit with a mol. wt. of 63000.
These conclusions are in agreement with the work of Grose et al. (1984) who described a disulphide-linked glycoprotein gp140 with gp66 as cleavage product. A second cleavage product with a mol. wt. of 128000 was also noted by them; however, the major cleavage product was gp66. Murine monoclonal antibodies reactive with gp140 were used to demonstrate its presence on infected cell membranes using indirect immunofluorescence assay.

Based on the immunoprecipitation technique, the IgG response to VZV polypeptides in varied clinical circumstances was determined. Antibodies to four glycoproteins with mol. wt. of 105K, 53K, 45K and 170K, resolved as 63K in reducing gels [170(63)K], developed in varicella convalescence. Minor bands in the profile indicated a weak response to the 93K and 81K glycoproteins. The major primary response in all six sera studied was to the disulphide-linked protein. Weigle & Grose (1984) noted antibodies to a similar array of glycoproteins within 1 to 2 months of varicella. We noted that antibodies to 170(63)K, 105K, 53K and 45K glycoproteins persisted for many decades following varicella.

Similar antibodies could not be detected in the majority of acute zoster sera. However, higher levels subsequently developed to the same four viral glycoproteins and in addition to the 93K and 81K glycoproteins. These antibodies persisted for 3 to 15 years following zoster as for varicella. In both instances antibody to the disulphide-linked glycoprotein occurred with greater frequency than antibody to those with mol. wt. of 105K, 53K and 45K. Low levels of antibodies to the disulphide-linked glycoprotein in quiescence may be a marker of susceptibility to reactivation.

In the group of patients with disseminated zoster, significantly lower levels of antibody ($P \leq 0.01$) to all the prominent antigens were noted when compared with normal zoster patients. Sera were time-matched: the mean number of days from onset was 6 days for the disseminated group whilst for the normal patients it was 6-5 days. One patient with gastric zoster and severe dissemination developed normal levels of antibody to all six characterized glycopeptides several days later than the control group. Absence of detectable antibodies at the 6th day from onset was the true indicator of risk in this patient. However, in severe infections immune complexes associated with excess circulating antigen may account for poor antibody detection.

Studies using monoclonal antibodies against VZV glycoproteins have established a non-complement-mediated neutralizing epitope associated with gp118 (Grose et al., 1983), corresponding to the 105K glycoprotein in the current study. Characterization of the biological functions of antibodies directed against the glycoproteins with mol. wt. 170K, 93K, 53K and 45K would elucidate their role in antibody-mediated recovery from infection. Isolation and characterization of the 170K and 105K glycoproteins could lead to their use in subunit vaccines, which might lead to more effective prevention and control of infection in those at risk from severe disease.

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REFERENCES


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