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Immune Response After Exposure to Varicella Zoster Virus: Characterization of Virus-Specific Antibodies and Their Corresponding Antigens

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Fourteen varicella zoster virus antigens were identified that induce antibodies during primary and recurrent infections. These antigens, which included the major nucleocapsid polypeptide (molecular weight, 155,000) and three glycoproteins (molecular weights, 130,000, 88,000, and 60,000, respectively) plus a number of minor antigens, were identified in radioimmunoprecipitation assays, using [^{35}S]methionine-labeled extracts of cells infected with varicella zoster virus and sera from patients with primary and recurrent viral infections. No significant and reproducible differences were observed between early convalescent sera from cases with natural chicken pox and sera from cases with zoster in their ability to react with viral antigens. Sera that were taken many years after episodes of chicken pox still retained their ability to react with the major viral antigens.

Varicella-zoster virus (VZV), in common with other herpesviruses, causes primary infections (varicella or chicken pox) and occasionally a recurrent infection, herpes zoster, usually seen in aging adults (13, 21). The immune mechanisms responsible for the prevention of and the recovery from primary and recurrent infections are poorly understood. Such understanding requires the characterization of viral antigens, especially those at the surface of infected cells that interact with the immune system.

Growth of VZV in tissue culture is limited, and the virus remains cell associated. This makes it very difficult to obtain sufficient quantities of purified virions for analyses and to assess their degree of purity. A recent report (22) described the purification of VZV and the analysis of its constituent polypeptides by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Although at least 31 polypeptides were identified, only 16 polypeptides were found in the precipitate obtained by reacting purified virions with immune serum.

The purpose of this work was to characterize those viral antigens that induce significant levels of antibodies in individuals with primary and recurrent VZV infections. Antigens and antibodies were characterized by immunoprecipitation assays as described previously (14). Extracts of [^{35}S]methionine-labeled WI-38 cells infected with VZV were reacted with a number of sera, and precipitated viral antigens were separated by SDS-PAGE and visualized by fluorography.

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MATERIALS AND METHODS

Medium, buffers, and solutions. EBME-FC10 medium is Eagle basal metabolic medium with Earle salts plus 10% fetal calf serum. NET buffer consists of 0.05 M tris(hydroxymethyl)aminomethane-0.005 M ethylenediaminetetraacetate-0.15 M NaCl-0.1 mM phenylmethylsulfonyl fluoride (pH 7.2), NET-T buffer is NET buffer containing 0.05% Triton X-100, and TE buffer consists of 0.01 M tris(hydroxymethyl)aminomethane-0.0025 M ethylenediaminetetraacetate (pH 7.5). Phosphate-buffered saline (PBS) consists of 0.04 M sodium phosphate-0.15 M NaCl (pH 7.2), and PBS-KCl is PBS with 0.005 M KCl. SP buffer consists of 0.01 M Tris (pH 6.8)-2% SDS-2% β-mercaptoethanol-20% glycerol-0.02% bromophenol blue. Five or 20% (wt/vol) sucrose was prepared in 0.05 M tris(hydroxymethyl)aminomethane-1 M NaCl-0.01 M ethylenediamine-tetraacetate-0.15% Sarcosyl (pH 7.5).

Cells. Human diploid fibroblasts (WI-38) at 26 to 29 population doublings were grown in EBME-FC10 medium.

Virus strains and sera. The strains of VZV were isolated from vesicle fluids obtained from clinical cases of varicella and grown in WI-38 cells (Table 1). The strains were identified at the third or fourth passage level, using cell-free virus obtained by disruption of the cells (2, 4), in plaque reduction neutralization tests. Paired acute and convalescent human sera obtained from clinical cases of varicella were used as well as horse hyperimmune serum made with a heterologous strain of VZV. All of the isolates were negative when tested for pleuropneumonia-like organisms and other extraneous agents.

Sera were obtained from cases with clinical varicella or zoster and from normal seropositive and -negative adults (Table 2).

Virus infection. Seed stocks of virus-infected cells were added to confluent monolayers of WI-38 cells (48 h after planting) at a ratio of 1:10 or 1:20 (infected to

TABLE 1. *VZV strains used*

VZV strain	Date of vesicle fluid	Passage level[a]
KMcC	4/11/66	4
KMcC	4/11/66	46
AW	4/09/66	5
AW	4/09/66	26
LB	1/11/79	28
RH	4/01/68	28

[a] All strains were passaged in WI-38 cells.

TABLE 2. *Sera used for immunoprecipitation of VZV polypeptides*

Serum designation	IAHA[a] titer	Description
V1-A	<2	Acute serum from child with varicella
V1-C	≥512	Early convalescent serum from child with varicella (6 wk after rash)
V2-C	≥512	Early convalescent serum from child with varicella (5 wk after rash)
79187	512	Early convalescent serum from child with varicella (2 wk after rash)
79058	1,024	Early convalescent serum from adult with varicella
V3	≥1,024	Early convalescent serum from adult with zoster
79100	512	Early convalescent serum from adult with zoster (14 days after shingles)
MSD-2	256	Serum from adult with long past chicken pox
MSD-4	<2	Serum from adult with long past chicken pox
MSD-14	<2	Serum from adult with long past chicken pox
HSV-AC	256	Acute serum from child with primary oral herpetic infection (HSV-specific neutralizing titer, <16)
HSV-CONV	512	Convalescent serum from above child (HSV-specific neutralizing titer, 64)

[a] Immune adherence hemagglutination assay (23).

uninfected cells) and incubated at 36°C. Cytopathogenicity, typical of varicella, affecting 30 to 60% of the cell sheet was observed in these cultures after approximately 72 h.

Labeling of infected cells. WI-38 cells were infected with VZV as described above, and after 72 h they were trypsinized. These cells were combined with freshly trypsinized uninfected WI-38 cells at a ratio of 1:4, replanted in 10-cm petri dishes (5×10^6 cells per dish) in EBME-FC10, and incubated at 36°C in a CO_2 incubator (17). When cytopathology involving approximately 50% of the culture was observed (generally 24 h later), the medium was removed and the cells were washed twice with PBS followed by the addition of 5 ml of medium containing 0.45 mg of methionine per liter (3% of the normal methionine concentration), nonessential amino acids, and 2% dialyzed fetal calf serum. One hour later [^{35}S]methionine (400 Ci/mmol; Amersham Corp.) was added to 30 μCi/ml, and the cells were incubated at 37°C for 16 h. After the addition of 5 ml of complete medium for 1 h, cells were washed with PBS and removed from the surface of the petri dish with 0.02% ethylenediaminetetraacetate and 0.05% $NaHCO_3$ in PBS-KCl containing 0.1 mM *N*-α-*p*-tosyl-L-lysine-chloromethyl ketone to inhibit proteases. Cells were pelleted, washed once in PBS, divided into six samples per petri dish (approximately 10^6 cells per sample), pelleted, and stored at −70°C. Unlabeled uninfected cells were also harvested with ethylenediaminetetraacetate and stored as two pellets per 10-cm petri dish (ca. 3×10^6 cells per sample).

Cells were labeled with D-[*U*-^{14}C]glucosamine (10 μCi/ml; >200 mCi/mmol; Amersham Corp.) by incubating them for 16 h in Eagle basal medium containing 20% of the normal glucose concentration and 2% dialyzed fetal calf serum. They were harvested and stored as described above.

Immunoprecipitation. Protein A-bearing staphylococcus immunoadsorbant (IgGsorb) was bought from the Enzyme Center, Inc. (Boston, Mass.). It was reconstituted to yield a 10% (vol/vol) suspension and kept at −70°C in 2-ml aliquots. Before use, the bacteria were washed as described (12, 14) and resuspended in NET-T. Frozen cell pellets (10^6 ^{35}S-labeled infected cells or 3×10^6 unlabeled uninfected cells) were suspended in 200 μl of NET buffer. Triton X-100 and deoxycholate were each added to 1%, and the suspension was kept at 4°C for 15 min. Nuclei were removed by centrifugation at $700 \times g$ for 6 min, and the supernatant was preadsorbed for 60 min at 22°C with 20 μl of normal rabbit serum, followed by the addition of 100 μl of protein A-bearing staphylococcus (20%, vol/vol) for 15 min at 22°C. This mixture was centrifuged at $6,500 \times g$ for 2 min, and the pellet was discarded. Samples, 20 μl, of diluted serum (generally 1:25) were preadsorbed for 15 min at 22°C with 20 μl of extract prepared from uninfected, unlabeled WI-38 cells. To this was added 20 μl of labeled extract (preadsorbed with normal rabbit serum) and this mixture was incubated for 60 min at 22°C, followed by the addition of 50 μl of protein A-bearing staphylococcus suspension (10% vol/vol). After incubation for 30 min at 22°C, complexes were pelleted at $6,500 \times g$ for 2 min and washed three times with 1.0 ml of NET-T buffer. The final pellet was resuspended in 70 μl of SP buffer and placed in boiling water for 3 min to dissociate the antigen-antibody complexes, the protein A-bearing staphylococcus was removed by centrifugation, and supernatants were applied to SDS-polyacrylamide gels.

Preparation of nuclear extracts. The nuclear pellet (see above) was washed with NET-T, resuspended in 20 μl of NET buffer containing 0.1% SDS, and sonicated twice for 20 s with a Kontes sonicator. The sonicated material was diluted to 100 μl with NET buffer and preadsorbed with normal rabbit serum as described above.

SDS-PAGE. Samples were electrophoresed in 10% discontinuous slab gels for 5 h at 110 V (15). Gels were fixed for 30 min in a mixture of methanol, acetic acid, and water (5:1:5) and soaked overnight in 7% acetic acid. Next, the gels were impregnated with 2,5-diphenyloxazole (22.4 g per 100 ml of dimethyl sulfoxide rinsed with water, dried, and exposed to Kodak Royal X-Omat film (for details, see reference 1). Molecular weight markers (Pharmacia Fine Chemical, Inc.) were

phosphorylase *b* (94,000), albumin (67,000), ovalbumin (43,000), carbonic anydrase (30,000), and trypsin inhibitor (20,100). They were labeled with ^{14}C-acetic anhydride by resuspending the contents of a Pharmacia vial in 1 ml of phosphate buffer (0.3 M; pH 7.2) followed by the addition of 500 μCi of ^{14}C-labeled acetic anhydride (60 to 120 mCi/mmol; Amersham Corp). The mixture was incubated for 30 min at room temperature, and labeled proteins were lyophilized after their purification by chromatography on a P-10 column (Pharmacia Fine Chemicals). Molecular weights of 100,000 and higher were estimated relative to the herpes simplex virus type 1 (HSV-1) nucleocapsid polypeptide and the A and B glycopolypeptides (19, 20; Zweerink and Stanton, submitted for publication).

Lectin-affinity chromatography. Cell extracts were fractionated on columns with the lectin from *Lens culinaris* coupled to Sepharose 4B. Lectin (Boehringer Mannheim Corp.), 30 mg, was reacted with 4 g of cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals), and columns (made in 3-ml syringes) with a volume (wet material) of approximately 0.5 ml were run at room temperature with a flow rate of 12 ml/h. [^{35}S]methionine-labeled infected cells (approximately 2×10^6) were suspended in 0.5 ml of NET buffer plus 1% Triton X-100. Nuclei were removed, and the cytoplasmic extract was applied to the column. First the column was washed with NET-T buffer adsorbed material was eluted with the same buffer containing 10 mg each of glucose and α-methyl-D-mannoside per ml (both carbohydrates were used for more efficient elution) or 3 M NH_4SCN. Fractions of 1.2 ml were collected and 20-μl aliquots were counted in Aquasol (New England Nuclear Corp.).

Isolation of VZV nucleocapsids. Infected cells were labeled overnight with ^{32}P (carrier-free; Amersham Corp.) at 50 μCi/ml in phosphate-free Eagle basal medium or with [^{35}S]methionine as described above. The supernatant was removed, and the cells were washed once with PBS and scraped with a rubber policeman into PBS. They were pelleted and washed once with PBS. The pellet (approximately 10^7 cells) was suspended in 1 ml of TE buffer before the addition of Triton X-100 to 1% and ribonuclease T_1 (50 U; Calbiochem). After 10 min in ice, nuclei were removed (1,500 rpm for 5 min), the cytoplasmic extract was kept at room temperature for 10 min, and Sarcosyl was added to 0.15%. The sample was placed on a 10-ml 5 to 20% sucrose gradient that had been layered over a 1-ml cushion of CsCl (ρ = 1.5 g/cm^3) in an SW41 tube. The gradient was centrifuged for 3 h at 35,000 rpm and 20°C, fractions of 0.5 ml each were collected from the bottom of the tube, and the radioactivity in a 20-μl aliquot of each sample was determined by Cerenkov radiation (for ^{32}P) or in Aquasol (for [^{35}S]methionine). Fractions containing viral nucleocapsids were combined and dialyzed against NET buffer.

Electron microscopy. A drop of purified VZV nucleocapsids in NET buffer (see above) was placed for 1 h on carbon-coated 200-mesh copper grids. The buffer was removed, and the grids were negatively stained with 2% phosphotungstic acid (pH 6.2) and examined in a Philips EM300 electron microscope at magnifications of 20,000 and 50,000.

RESULTS

VZV polypeptides in infected cells. Often, intracellular virus-specific polypeptides can be identified by comparing electropherograms of radiolabeled polypeptides in infected and uninfected cells (9). This approach could not be used for VZV, since virus-specific polypeptide synthesis is not extensive and host-specific protein synthesis continues.

Therefore, VZV-specific polypeptides were analyzed by immunoprecipitation. Cytoplasmic extracts from [^{35}S]methionine-labeled uninfected WI-38 cells and WI-38 cells infected with VZV strain KMcC (passage 46) were incubated with acute and early convalescent sera from a child with clinical varicella, and precipitated antigens were analyzed by SDS-PAGE and fluorography.

Figure 1 shows that at least 14 polypeptides ranging in molecular weight from less than 20,000 to approximately 200,000 were precipitated from infected cell extracts with immune serum, but not with acute serum, and were not precipitated from uninfected cells. Polypeptide

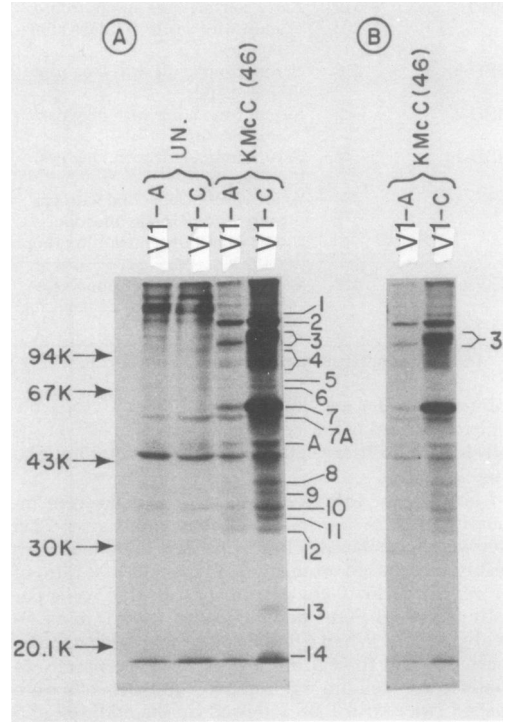

FIG. 1. *Fluorograms of polypeptides precipitated by acute (V1-A) and convalescent (V1-C) sera from cytoplasmic extracts of uninfected WI-38 cells (UN) or WI-38 cells infected with VZV KMcC (passage 46). (A) Film exposure, 4 days; (B) film exposure, 1 day.*

3 occasionally migrated as three separate bands (see Fig. 1B), and the material designated as polypeptide 4 always migrated as a broad band. Polypeptide 4 could represent either several polypeptide species or a single polypeptide species with variable carbohydrate side chains (see below). The polypeptide labeled "A" is an artifact caused by the accumulation of the immunoglobulin heavy chain in that region of the gel. Some precipitation of polypeptides 2 and 7 and the smallest of the three polypeptide 3 species by acute serum was often observed. This could be due to either nonspecific precipitation or the fact that acute serum contained low levels of antibodies against these polypeptides. Polypeptide 7A was precipitated from uninfected cells with both sera and from infected cells with control serum. However, precipitation was heaviest when infected cell extracts were reacted with immune serum; no further attempts were made to determine the origin of this polypeptide.

Characterization of viral polypeptides. Glycopolypeptides were identified by exposing infected cells to [^{14}C]glucosamine during viral growth. Figure 2, gel B, shows that this carbohydrate is incorporated into polypeptides 3, 4, and 7. The glycoprotein nature of these polypeptides was confirmed by fractionating cytoplasmic extracts of [^{35}S]methionine-labeled infected cells on a column with the lectin from *L. culinaris*. Three fractions were obtained: the unadsorbed material, adsorbed material that eluted with α-methyl-mannoside and glucose, and adsorbed material that eluted with 3 M NH_4SCN (fractions A, B, and C, respectively, in Fig. 3). Each fraction was reacted with immune serum, and the precipitated polypeptides were analyzed by SDS-PAGE. Figure 2, gel D, shows that the material in fraction B contained viral polypeptides 3 and 4 and a lesser amount of 7 and that fraction C was enriched for polypeptide 7. The unadsorbed material (Fig. 2, gel C) contained mainly polypeptides 2 and 4 and small amounts of polypeptides 3 and 7.

As will be shown below, polypeptide 2 is the major nucleocapsid component. Its presence in the lectin-adsorbed material is probably due to incomplete removal of glycoproteins from the nucleocapsids and binding of these complexes to the lectin. Complete dissociation of the glycoproteins (especially polypeptide 7) from the lectin requires strong denaturing conditions (3 M NH_4SCN). Failure of cell glycoprotein material to bind to the column (see Fig. 2, gel C) probably reflects heterogeneity in glycosylation.

The nucleocapsid polypeptides were identified after isolating nucleocapsids from the cytoplasm of infected cells by centrifugation in sucrose density gradients. For identification purposes

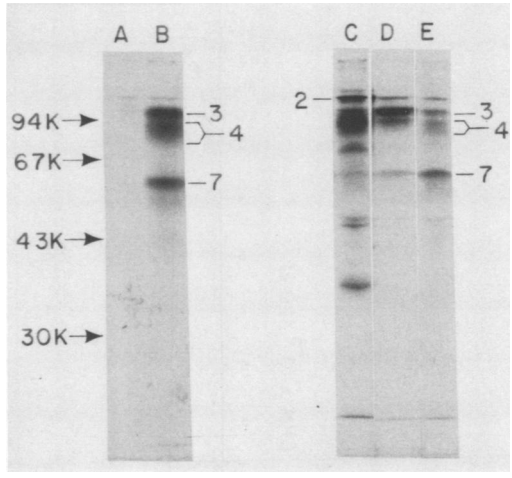

FIG. 2. *Fluorograms of VZV glycoproteins. (A) Cytoplasmic extract of WI-38 cells infected with VZV KMcC (passage 46) and labeled with [^{14}C]glucosamine was reacted with acute serum (V1-A); (B) same extract reacted with immune serum (V1-C); (C, D, and E) fractions A, B, and C from Fig. 3 (labeled with [^{35}S]methionine) reacted with immune serum (V1-C).*

viral deoxyribonucleic acid was labeled with ^{32}P. Most of the ^{32}P-labeled material that was associated with structures large enough to enter the gradient sedimented on top of the CsCl cushion (Fig. 4). By electron microscopy it was shown that this material consisted of nucleocapsids (Fig. 5). A small amount of radiolabeled material sedimented more slowly (see arrow in Fig. 4). This also is the position to which HSV nucleocapsids sedimented.

Nucleocapsid polypeptides were identified by labeling infected or uninfected WI-38 cells with [^{35}S]methionine, and the cytoplasmic extracts were fractionated by sedimentation in sucrose density gradients. The material at the interface between CsCl- and sucrose-containing viral nucleocapsids (see Fig. 5) was dialyzed against NET buffer and pelleted at 35,000 rpm for 3 h, and the polypeptide composition was determined by SDS-PAGE. Figure 6 (gel B) shows that polypeptide 2 was the major constituent of nucleocapsids; smaller amounts of the glycoproteins 3, 4, and 7 and the low-molecular-weight polypeptides 10, 12, and 14 were also found in cores. None of these polypeptides was present in fractionated cytoplasmic extract from uninfected cells (Fig. 6, gel A). Possibly glycoproteins were incompletely removed from the nucleocapsids by detergent treatment, whereas polypeptides 10, 12, and 14 could be minor-constituent nucleocapsid polypeptides. Nucleocapsid polypeptide 2 was the major viral antigen in isolated nuclei of infected cells (data not shown).

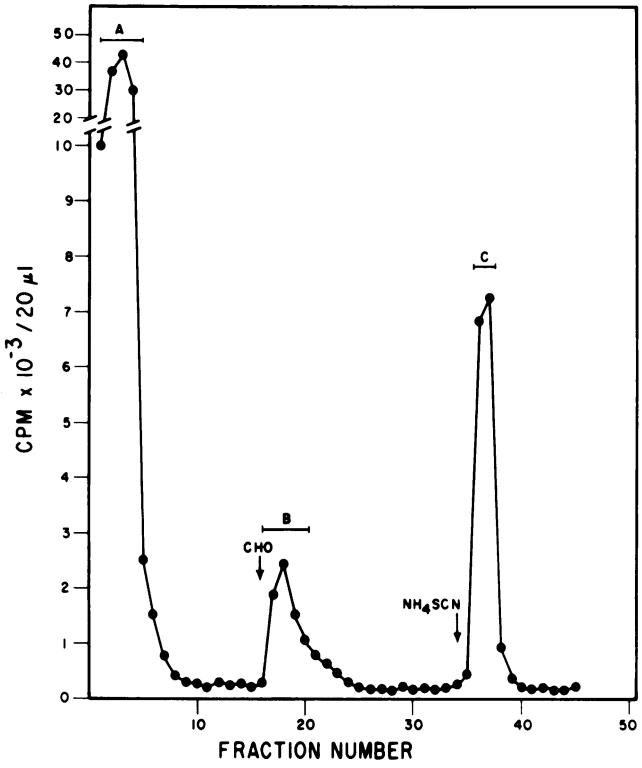

FIG. 3. *L. culinaris affinity chromatography of the cytoplasmic extract of WI-38 cells infected with VZV KMcC (passage 46) and labeled with [35S]methionine. (Peak A) Unadsorbed material; (peak B) material eluted with the carbohydrates (CHO) α-methyl-mannoside and glucose; (peak C) material eluted with 3 M NH4SCN.*

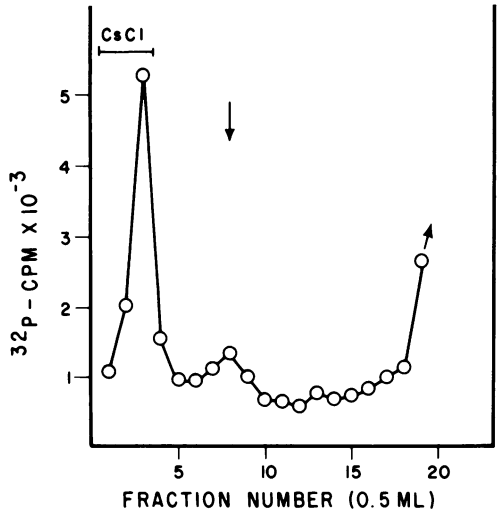

FIG. 4. *Isolation of VZV nucleocapsid. WI-38 cells infected with VZV KMcC (passage 46) were labeled with 32P, and the cytoplasmic extract was fractionated on a sucrose gradient that was layered over a cushion of CsCl, as described in the text.*

Coelectrophoresis of polypeptides precipitated from VZV-infected cells with those precipitated from cells infected with HSV-1 showed that the major nucleocapsid polypeptides of each virus migrated at the same rate, suggesting identical molecular weights. Similarly, VZV glycoprotein 3 coelectrophoresed with the major A and B HSV-1 glycoprotein complex (19, data not shown).

The properties of the VZV polypeptides and their molecular weights are summarized in Table 3.

Polypeptides in various VZV isolates. It has been reported for HSV that there are differences in the polypeptides (relative amounts and molecular weights) of the two serotypes (HSV-1 and HSV-2) and the various isolates within each serotype (5, 8). We investigated whether this was also the case for VZV. WI-38 cells were infected with four different VZV isolates, strains KMcC (passage 46), AW (passage 26), LB (passage 28), and RH (passage 28), and exposed to [35S]methionine. Viral polypeptides were precipitated with immune serum and analyzed by SDS-PAGE. Figure 7 shows no differences in

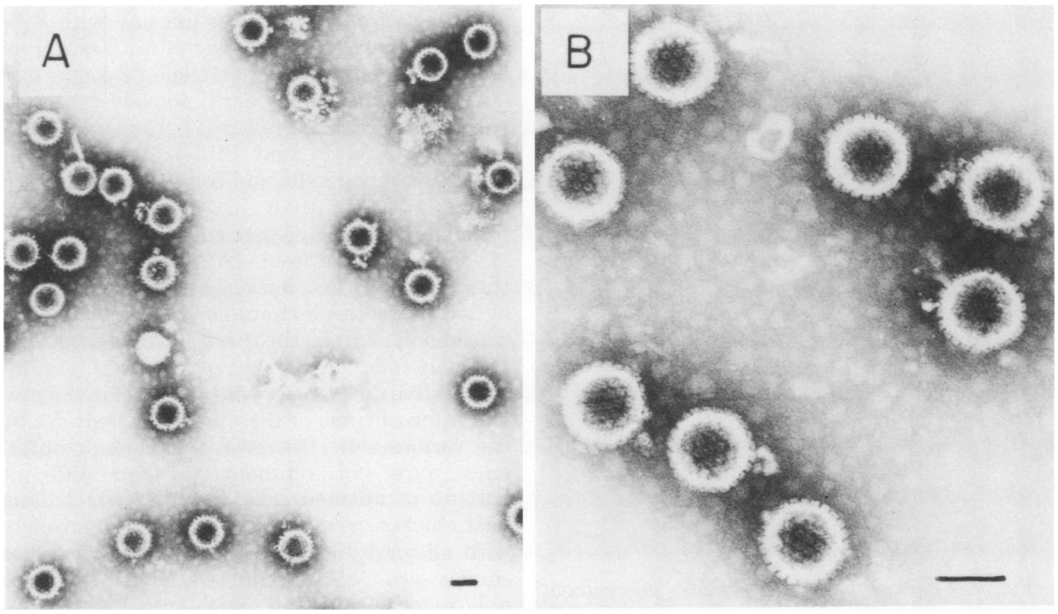

FIG. 5. *Electron micrograph of nucleocapsids isolated from the interface between CsCl and sucrose (see Fig. 4). (A) and (B) are two different magnifications. Bar, 100 nm.*

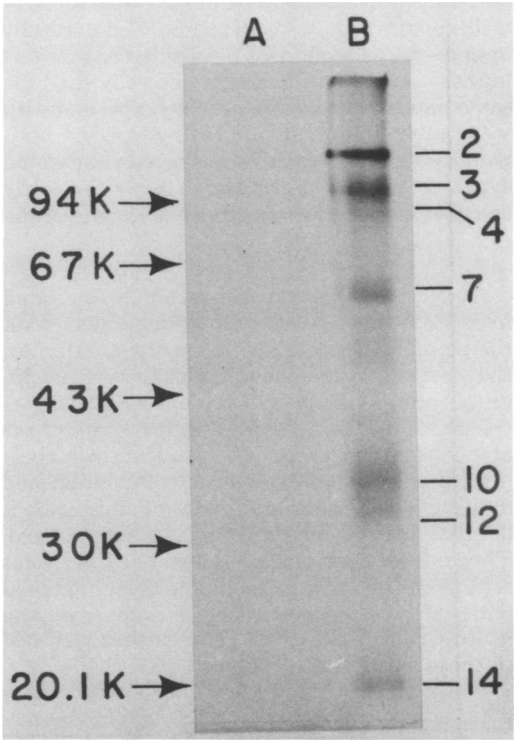

FIG. 6. *Fluorograms of polypeptides in VZV nucleocapsids that were isolated by sucrose-CsCl sedimentation (see text) from the cytoplasmic extract of [^{35}S]methionine-labeled WI-38 cells infected with VZV KMcC (passage 46). For comparison, uninfected cells were fractionated and analyzed in a similar fashion. (A) Uninfected cells; (B) infected cells.*

TABLE 3. *Summary of VZV polypeptides*

No.	Mol wt	Property
1	≥200,000	
2	155,000	Major nucleocapsid polypeptide
3	130,000	Glycoprotein
4	88,000	Glycoprotein
5	73,000	
6	67,000	
7	60,000	Glycoprotein
7a	57,000	Possibly host cell specific
8	39,000	
9	37,000	
10	34,500	Possibly minor nucleocapsid polypeptide
11	33,000	
12	31,500	Possibly minor nucleocapsid polypeptide
13	22,500	
14	17,500	Possibly minor nucleocapsid polypeptide

the migration rate or the relative amounts of the major polypeptides. Differences in the relative amounts of the minor polypeptides were observed, but these were not reproducible, and they were also observed with different preparations of the same isolate (cf. Fig. 1 and 7).

It was also shown that passaging of VZV in WI-38 cells did not alter the polypeptide pattern.

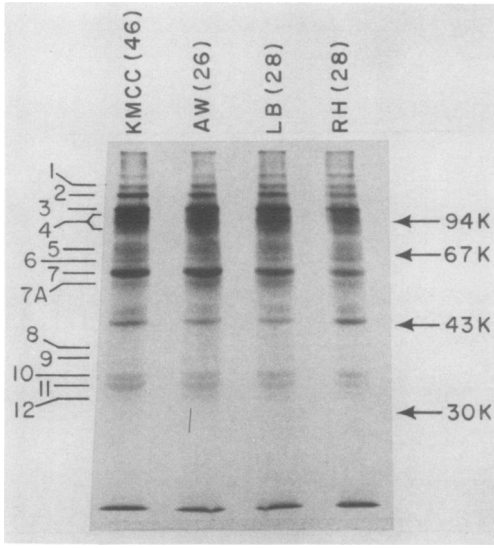

FIG. 7. *Fluorograms of polypeptides precipitated by immune serum (V1-C) from extracts of WI-38 cells infected with VZV KMcC (passage 46), AW (passage 26), LB (passage 28), and RH (passage 28).*

No differences were observed between VZV KMcC at passage levels 6 and 46 or VZV AW at passage levels 6 and 26 (data not shown).

Reactivity of various sera with VZV polypeptides. Immunoprecipitation analyses as used in this paper can be used to identify viral antigens in infected cells, or conversely, precipitated antigens can serve as indicators for specific antibodies in sera. This requires a minimum of macromolecular aggregates that consist of more than one polypeptide species in the extracts from infected cells. We have shown that such aggregates, if present, do not constitute a problem in extracts prepared from cells infected with HSV (Zweerink and Stanton, Infect. Immun., in press). The possible presence of aggregates in extracts of VZV-infected cells was investigated as follows. Extracts prepared as described in Materials and Methods were reacted with control serum and immune serum before and after centrifugation at 100,000 × *g* for 60 min, and the precipitated polypeptides were analyzed by SDS-PAGE and fluorography. The immunoprecipitation pattern of the minor polypeptides and the major polypeptides 2, 3, 4, and 7 was not affected by high-speed centrifugation except that there was a small, but reproducible, decrease in the amount of precipitated nucleocapsid polypeptide 2 (data not shown). This demonstrates that large structures such as incompletely solubilized virions are not present in sufficient quantities to influence these assays.

Extracts from WI-38 cells infected with VZV KMcC (passage 46) were reacted with sera from individuals with clinical varicella or zoster (see Table 2). These were early convalescent sera with high IAHA titers (≥512) from three children (V1-C V2-C, and 79157) and one adult (79058) with varicella and from two adults with zoster (V3 and 79100). In addition, three sera from adults with long past chicken pox (MSD-2, -4, and -14) were used. IAHA titers of two of these sera were low, but one (MSD-2) had a titer of 256. All sera precipitated the major nucleocapsid polypeptide, the three glycopolypeptides, and a number of minor polypeptides (Fig. 8). Qualitative differences were observed in the precipitation of some of the minor polypeptides by the various sera. However, these minor differenes were not reproducible with different batches of infected cells. Serum MSD-2 (long past chicken pox; IAHA, 256) reacted strongly with all viral antigens, whereas the two other similar sera (MSD-10 and -14; IAHA, <2) were only reactive with the major antigens (the nucleocapsid polypeptide and the three glycoproteins). Figure 8 also shows that acute and early convalescent sera from a child with a primary herpetic infection (increase in HSV-specific neutralizing antibodies, <1:16 to 1:64) and naturally immune to varicella (VZV-specific IAHA titers in these sera, 256 and 512, respectively) had the same pattern of reactivity in both sera and with VZV polypeptides. If herpesvirus-specific antibodies could react with VZV antigens, one would expect increased precipitation with the early convalescent serum compared with acute serum.

DISCUSSION

At least 14 polypeptides were precipitated from VZV-infected cells with immune sera. With the possible exception of one (polypeptide 7A), they were virus coded because they were precipitated preferentially by immune sera from extracts of infected cells. Sera in the acute phase of varicella were used for controls in these studies. It has been our experience (14; unpublished data) that these sera contain few if any antibodies that can be measured in the assays used. They offer the advantage (unless one has earlier sera from the same patients) of being matched with early convalescent sera. Results with the acute serum of V2-A (see Table 2) were identical to those with serum V1-A.

It is unlikely that any host cell-specific polypeptides were trapped by the protein A-bearing staphylococcus-antibody-antigen complexes. This was shown by reacting immune serum with a mixture of extracts from unlabeled infected cells and [^{35}S]methionine-labeled uninfected

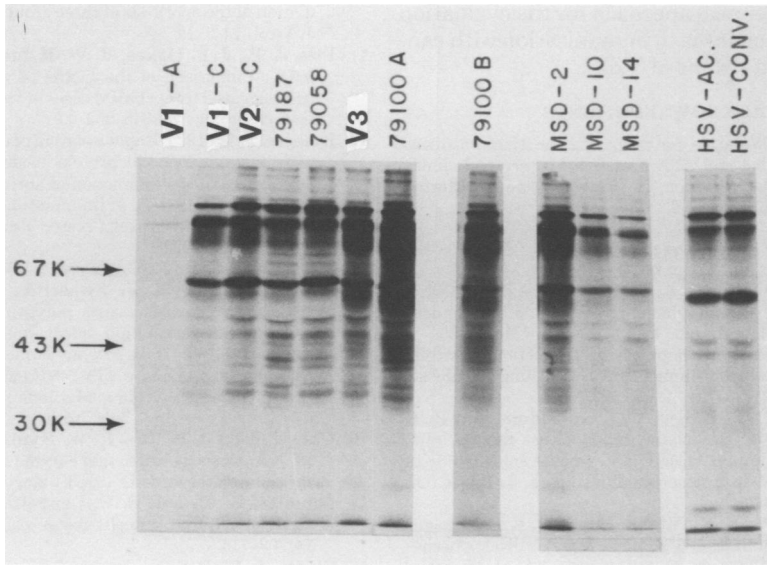

FIG. 8. *Fluorograms of polypeptides precipitated from WI-38 cells infected with VZV KMcC (passage 46) by sera from patients with chicken pox or zoster and individuals with long past chicken pox (see Table 2). HSV-AC and HSV-CONV are acute and convalescent sera, respectively, from a child with primary oral herpes lesions. 79100A and 79100B are the same gel but exposed for 3 and 1 days, respectively.*

cells. The precipitated radioactive polypeptides did not contain any of the virus-specific polypeptides that are indicated in Fig. 1 (data not shown). Four of the major precipitated polypeptides were characterized further: one was the nucleocapsid polypeptide; the other three were glycoproteins, presumably representing envelope antigens. They corresponded to glycoproteins described by Grose (7). A fourth glycoprotein (molecular weight, 45,000) described by Grose was not observed by us.

The molecular weight of VZV deoxyribonucleic acid has been reported to be 100×10^6 (11), with a coding capacity of 5×10^6 daltons of polypeptides. The total molecular weight of the VZV polypeptides in Table 2 is approximately 10^6, which is 20% of the maximum coding capacity. Several of the radioactive bands in the gels may have contained more than one polypeptide species (see, for example, polypeptide 3 in Fig. 1), and minor polypeptide species may have been overlooked. Furthermore, polypeptides that did not induce specific antibodies during natural infections will not be detected by the methods used in this paper. It is likely that VZV polypeptide synthesis is regulated as is the case for other herpes-viruses (10). [^{35}S]methionine was added to cultures late during infection at a time of moderate cytopathic effect, when only part of the viral genome was expressed. One might also expect differences in the extent to which viral replication had proceeded in the various cultures used for labeling, which would explain why different batches of infected cells varied somewhat in their content of precipitated viral polypeptides.

No differences in the relative amounts and the molecular weights of the major polypeptides in four different isolates of VZV were observed. Similar results were obtained for two viral strains that had been passaged 6 and 46 times, respectively, in WI-38 cells. This is in contrast to HSV, for which differences between viral isolates of the same serotype have been observed (5, 8). Stability between VZV isolates has also been observed at the level of its deoxyribonucleic acid: variations of the restriction endonuclease profiles of a number of isolates of both HSV serotypes have been observed repeatedly (3, 18), whereas such profiles are much more stable for isolates of VZV (16; our unpublished data).

Sera from patients with chicken pox (a primary infection) and herpes zoster (a recurrent infection) contained the same antibodies as judged by the immunoprecipitation assays, and sera from normal adults who presumably had had varicella during childhood still contained antibodies against the major nucleocapsid polypeptide and the three glycoproteins.

The identification of several viral glycoproteins that induce antibodies during primary infection and recurrences provides a rationale for selecting antigens to be included in a potential VZV subunit vaccine. Furthermore, it provides

an immunochemical approach for the evaluation of antibody responses to immunization with candidate vaccine strains of VZV.

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LITERATURE CITED

1. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
2. **Brunell, P. A.** 1967. Separation of infectious varicella-zoster virus from human embryonic lung fibroblasts. Virology **31**:723–734.
3. **Buchman, T. G., B. Roizman, G. Adams, and B. H. Stover.** 1978. Restriction endonuclease fingerprinting of herpes simplex virus DNA: a novel epidemiological tool applied to a nosocomial outbreak. J. Infect. Dis. **138**:488–498.
4. **Calnek, B. W., S. B. Hitchner, and H. K. Adldinger.** 1970. Lyophilization of cell-free Marek's disease herpesvirus and a herpesvirus from turkeys. Appl. Microbiol. **20**:723–726.
5. **Cassai, E. N., M. Sarmiento, and P. G. Spear.** 1975. Comparison of the virion proteins specified by herpes simplex virus types 1 and 2. J. Virol. **16**:1327–1331.
6. **Gibson, W., and B. Roizman.** 1972. Proteins specified by herpes simplex virus. VIII. Characterization and composition of multiple capsid forms of subtypes 1 and 2. J. Virol. **10**:1044–1052.
7. **Grose, C.** 1980. The synthesis of glycoproteins in human melanoma cells infected with varicella-zoster virus. Virology **101**:1–9.
8. **Heine, J. W., R. W. Honess, E. Cassai, and B. Roizman.** 1974. Proteins specified by herpes simplex virus. XII. The virion polypeptides of type 1 strains. J. Virol. **14**:640–651.
9. **Honess, R. W., and B. Roizman.** 1973. Proteins specified by herpes simplex virus. XI. Identification and relative molar rates of synthesis of structural and nonstuctural herpes virus polypeptides in the infected cell. J. Virol. **12**:1347–1365.
10. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. **14**:8–19.
11. **Iltis, J. P., J. E. Oakes, R. W. Hyman, and F. Rapp.** 1977. Comparison of the DNAs of varicella-zoster viruses isolated from clinical cases of varicella and herpes zoster. Virology **82**:345–352.
12. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115**:1617–1624.
13. **Luby, J. P.** 1973. Varicella-zoster virus. J. Invest. Dermatol. **61**:212–222.
14. **Machamer, C. E., E. C. Hayes, S. D. Gollobin, L. K. Westfall, and H. J. Zweerink.** 1980. Antibodies against the measles matrix polypeptide after clinical infection and vaccination. Infect. Immun. **27**:817–825.
15. **Maizel, J. V., Jr.** 1971. Polyacrylamide gel electrophoresis of viral proteins, p. 179–246. *In* C. K. Maramorosch and H. Koprowsky (ed.), Methods in virology, vol. 5. Academic Press, Inc., New York.
16. **Oakes, J. E., J. P. Iltis, R. W. Hyman, and F. Rapp.** 1977. Analysis by restriction enzyme cleavage of human varicella-zoster virus DNAs. Virology **82**:353–361.
17. **Schmidt, N. J., and E. H. Lennette.** 1976. Improved yields of cell-free varicella-zoster virus. Infect. Immun. **14**:709–715.
18. **Skare, J., W. P. Summers, and W. C. Summers.** 1975. Structure and function of herpesvirus genomes. I. Comparison of five HSV-1 and two HSV-2 strains by cleavage of their DNA with *Eco R* I restriction endonuclease. J. Virol. **15**:726–732.
19. **Spear, P. G.,** 1976. Membrane proteins specified by herpes simplex viruses. I. Identification of four glycoprotein precursors and their products in type 1-infected cells. J. Virol. **17**:991–1008.
20. **Spear, P. G., and B. Roizman.** 1972. Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpes virion. J. Virol. **9**:143–159.
21. **Taylor-Robinson, D., and A. E. Caunt.** 1972. Varicella virus. Virol. Monogr. **12**:27.
22. **Wolff, M. H.** 1978. The proteins of varicella-zoster virus. Med. Microbiol. Immunol. **166**:21–28.
23. **Yamada, A., S. Ogino, Y. Asano, T. Otsuka, M. Takahashi, K. Baba, and H. Yabuuchi.** 1979. Comparison of 4 serological tests—complement fixation, neutralization, fluorescent antibody to membrane antigen and immune adherence hemagglutination—for assay of antibody to varicella-zoster (V-Z) virus. Biken J. **22**:55–60.