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Immunogenic Glycoproteins of Laboratory and Vaccine Strains of Varicella-Zoster Virus

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High-titered antisera were prepared in guinea pigs and rabbits against two strains of varicella-zoster virus (VZV): VZV-32, a low-passage laboratory strain, and VZV-Oka, a vaccine strain attenuated by passage in both human and guinea pig embryo cells. When the animal VZV-immune sera, as well as a human zoster serum, were used to precipitate radiolabeled glycoproteins from VZV-infected cells and the immune precipitates were analyzed by polyacrylamide gel electrophoresis and fluorography, it was observed that cell cultures infected with either strain had similar electrophoretic profiles containing major glycoproteins of approximate molecular weights 62,000, 98,000, and 118,000. A prominent high-molecular-weight (~150,000) nonglycosylated polypeptide was identified in both strains also. These determinants were demonstrable by both indirect (staphylococcal protein A-antibody adsorbent) and direct immunoprecipitation, as long as VZV-immune sera with an antibody titer ≥1:128 were used. Further analysis of individual caviid VZV antisera demonstrated some heterogeneity which appeared to be related to the method of immunization rather than the level of virus-specific antibody. VZV extracts emulsified with complete Freund adjuvant elicited an antibody response to all major immunogenic viral glycoproteins, whereas guinea pigs inoculated with virus alone during the primary immunization initially produced VZV antibody which failed to precipitate the highest-molecular-weight glycoprotein (gp118). Thus, Freund-type adjuvants promoted the maturation of the humoral immune response after VZV immunization in outbred guinea pigs.

Clinical trials with live varicella-zoster virus (VZV) vaccines are underway in Japan (2) and Europe (12) and are being initiated in the United States. The immunization programs have focused attention in particular on one of the candidate VZV vaccines, designated Oka strain (24), because of an attenuation history which included 12 subcultivations in guinea pig embryo cells. At a National Institutes of Health Workshop on Experimental Herpesvirus Vaccines (sponsored by the Bureau of Biologics, Food and Drug Administration, National Cancer Institute, and National Institute of Allergy and Infectious Diseases, February 1979, Bethesda, Md.), several questions were raised about possible antigenic variation between this and other VZV strains, yet little information is available because the molecular structure of the virus has not been well characterized.

Recent studies of VZV infection in human melanoma cell (HMC) monolayers defined the conditions under which an exponential increase in virus yield was achieved as cytopathic effect (CPE) increased from approximately 50 to nearly 100% (8). Addition of radioactive sugars or amino acids to the culture medium during the late phase of the in vitro infection demonstrated a reduction in the synthesis of host cell proteins and the concomitant appearance of infected-cell-specific (ICS) proteins and glycoproteins (7). To expand upon these observations, we have produced antisera to low-passage laboratory and vaccine VZV strains in guinea pigs and rabbits and have used the techniques of direct and indirect immunoprecipitation to identify mainly the immunogenic glycoproteins encoded by the VZV strains of different origins. Because of renewed interest in experimental VZV infection of guinea pigs (17, 26), we have also defined by serology and immunoprecipitation the virus-specific caviid antibody response after VZV immunization.

MATERIALS AND METHODS

Cell cultures. Monolayer cultures of HMC were grown in Eagle minimal essential medium supplemented with 2 mM glutamine, 1% nonessential amino acids, penicillin (100 U/ml), streptomycin (100 μg/ml), and 8% fetal bovine serum (MEM-FBS) (8). Primary guinea pig embryo cell cultures were prepared by mincing and trypsinizing whole guinea pig embryos 1 to 2 cm in length as recently described (B. J. Edmond, C. Grose, and P. A. Brunell, submitted for

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publication). These cells were grown in MEM-FBS and subcultivated at a 1:3 split ratio no more than five times.

Origin of virus strains. The VZV-32 strain of VZV (8), which was isolated from the vesicular fluid of an 11-year-old boy with chickenpox, was serially propagated at 32°C in HMC cultures by passage of trypsin-dispersed infected cells. The vaccine strain (VZV-Oka), which also originated from a child with chickenpox, was attenuated by subcultivation in human embryo lung cells (X11), guinea pig embryo cells (X12), and the WI-38 line of human diploid cells (X6) (24). The latter strain was further passaged in either guinea pig embryo or HMC monolayers before use in some of the experiments described here.

Infection of cells and isotopic labeling. Nearly confluent HMC monolayers were infected at a ratio of one inoculum cell to eight uninfected cells; the inoculum consisted of trypsin-dispersed cells from a VZV-infected monolayer with advanced CPE. When CPE reached 50% in the newly infected monolayers, the culture medium was replaced with MEM-FBS containing 10 μCi of tritiated glucosamine or fucose per ml. After an additional incubation for 24 h at 32°C, during which time CPE advanced to greater than 90%, the cultures were harvested with a rubber policeman. In one experiment, VZV-infected monolayers were incubated in the presence of MEM-FBS deficient in methionine but substituted with [^{35}S]methionine (5 μCi/ml).

Human and rabbit VZV-immune sera. A human serum with a high VZV antibody titer (≥1:512) was obtained from an otherwise healthy college student with recent herpes zoster (7). The preparation of a rabbit VZV-immune serum has been previously described (10). Briefly, an extract prepared from sonically disrupted HMC monolayers infected with VZV-32 was emulsified at a 1:1 ratio with complete Freund adjuvant (Difco Laboratories) and injected into the footpad of a male New Zealand white rabbit. After 1 month the rabbit was given a booster inoculation of the same material. The rabbit serum drawn 1 week later inhibited VZV plaque formation by 50% at a dilution of 1:800. Rabbit antiserum to the vaccine strain was produced in the same manner and possessed VZV-neutralizing activity at a titer of 1:128. Both rabbit VZV-immune sera were precipitated with ammonium sulfate (pH 7.0); the globulin fractions were dialyzed against 0.01 M phosphate-buffered saline and adsorbed overnight with uninfected cells to remove anticell activity. The production of three VZV-immune sera in guinea pigs is described in Results. Because of the limited quantities available, the human serum and the guinea pig sera were not salt fractionated. The VZV serological tests (plaque reduction neutralization and fluorescent antibody to membrane antigen) were performed as previously described (9). Fluorescein-conjugated goat antisera to human, rabbit, and guinea pig immunoglobulin G were purchased from Cappel Laboratories, Cochranville, Pa.

Immunoprecipitation. Virus-infected HMC monolayers (75 cm^2) were washed twice with cold 0.01 M phosphate-buffered saline and dislodged with a rubber policeman into 0.06 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 6.8). Cell lysates were solubilized by the addition of the detergents Nonidet P-40 and deoxycholate to final concentrations of 1% (vol/vol) and 1% (wt/vol), respectively. After an incubation period of 15 min at room temperature, cellular debris was removed by centrifugation at 1,000 × *g* for 10 min. The cell extracts were placed in 2-ml adapters and centrifuged at 85,000 × *g* for 1 h in a type 50 rotor. Portions (0.2 ml) of the high-speed supernatants were mixed with equal volumes of VZV antisera, and the immune complexes were sedimented as previously described (7).

In later experiments, the sensitivity of the immunoprecipitation technique was enhanced by the addition of staphylococcal protein A which binds more than 90% of human, rabbit, or guinea pig immunoglobulin G via the Fc receptor (13). Solubilized VZV lysates (100 μl) were incubated at ambient temperature with VZV antisera for 30 min. The initial antigen concentration was diluted 1:3 with the addition of 200 μl of modified Schwyzer buffer containing final concentrations of 1% Nonidet P-40 and deoxycholate (19). After the final saline concentration was adjusted to 200 mM, the samples were incubated at 4°C for 16 to 18 h with agitation. Incubation was continued for an additional 3 h after the addition of 110 μl of preswollen protein A-sepharose CL-4B beads (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). The beads were washed twice (10 times the final volume) with 0.1 M Tris–0.5 M LiCl (pH 8.8) before sedimentation at 2,000 rpm for 10 min. Immune complexes were eluted by incubating the washed beads with 110 μl of sample buffer (7). Subsequently, the beads were removed by centrifugation at 2,000 rpm for 10 min. The radioactive supernatants were stored at −20°C.

Polyacrylamide gel electrophoresis and fluorography. Polyacrylamide slab gel electrophoresis in Tris-glycine buffer (pH 8.1) containing 0.1% sodium dodecyl sulfate was used to analyze the immune precipitates (16). The separation gels contained 8 or 10% acrylamide, and the stacking gel was composed of 4% acrylamide. The monomer was cross-linked with either 2.7% methylenebisacrylamide (MBA) or an equal amount of *N,N*-diallyltartardiamide (DATD). The radioactive samples were solubilized and subjected to electrophoresis as previously described (7). After completion of the electrophoresis, the slab gels were suffused with the scintillant diphenoxyoxazole dissolved in dimethyl sulfoxide to enhance the detection of tritium-labeled proteins (4). Exposure times of the dried gel to Kodak XR-5 or XRP-5 film ranged from 4 days to 3 weeks. Some of the films were analyzed with a soft laser scanning densitometer (LKB Instruments Inc., Rockville, Md.).

Chemicals and isotopes. The radiochemicals [6-^{3}H]glucosamine (specific activity, 38 Ci/mmol), [6-^{3}H]fucose (specific activity, 26 Ci/mmol), [5,6-^{3}H]fucose (specific activity, 56 Ci/mmol), and [^{35}S]methionine (specific activity, 1,350 Ci/mmol) were purchased from Amersham Corp., Arlington Heights, Ill., or New England Nuclear Corp., Boston, Mass. Eastman Organic Chemicals (Rochester, N.Y.) supplied diphenoxyoxazole and the reagents for polyacrylamide gel electrophoresis. The molecular weight standards were purchased from Sigma Chemical Co., St. Louis, Mo., and Bio-Rad Laboratories, Richmond, Calif. Nonidet

P-40 was obtained from Particle Data Laboratories, Elmhurst, Ill.; dimethyl sulfoxide and deoxycholate were obtained from Fisher Chemical (Fair Lawn, N.J.)

RESULTS

Guinea pig VZV-immune sera. Each of three infant Hartley strain guinea pigs was inoculated subcutaneously with one of the following virus strains: (i) a cell-free preparation of vaccine virus (VZV-Oka); (ii) a cell-free preparation of vaccine virus which had been further passaged 12 times in guinea pig embryo cells, and (iii) a trypsin-dispersed suspension of HMC infected with VZV-32 strain (passage 14). Initial antibody titers (S_1 sera) by indirect immunofluorescence ranged from 1:4 to 1:8. Each animal was given a second inoculation in the footpad with the homologous VZV strain mixed 1:1 with complete Freund adjuvant. Seven to 10 days after the antigenic boost, the guinea pigs demonstrated 16- to 32-fold rises in VZV-specific antibody titer in their S_2 sera (Table 1). When uninfected human cells were used as a control in the fluorescence assay, titers of 1:32 or 1:64 were found in sera from guinea pigs which had been immunized with virus prepared from VZV-infected human cells. In each instance, however, the titer of anticell antibody was at least fourfold lower than that of virus-specific antibody (Table 1). In contrast to the above results, no anticell activity was detected in the VZV-immune serum obtained from guinea pig ii, which had been inoculated with VZV serially propagated in guinea pig embryo cells to remove human cell determinants.

Direct immunoprecipitation of VZV-32-infected cell extracts. Cells infected with VZV-32 were incubated with medium containing [^{3}H]glucosamine during the late phase of infection. A detergent-solubilized extract was mixed with equal amounts of each of three VZV antisera: (i) human zoster serum, (ii) rabbit antiserum to VZV-32, or (iii) guinea pig antiserum to VZV-Oka vaccine further passaged in guinea pig embryo cells. Figure 1 shows the electrophoretic patterns of the three immune precipitates; the

TABLE 1. *Peak antibody response of guinea pigs to VZV immunization*

Guinea pig no.	Immunizing agent[a]	FAMA[b] titer		NT[c] titer
		Infected cells	Uninfected cells	
i	VZV-Oka	1:512	1:32	1:256
ii	VZV-Oka P12 in GPEC	1:256	<1:2	1:128
iii	VZV-32	1:256	1:32	1:128

[a] See text for further description of VZV strains; GPEC, guinea pig embryo cells.
[b] Indirect fluorescence assay to detect antibody against membrane antigen (9).
[c] VZV neutralization assay (9).

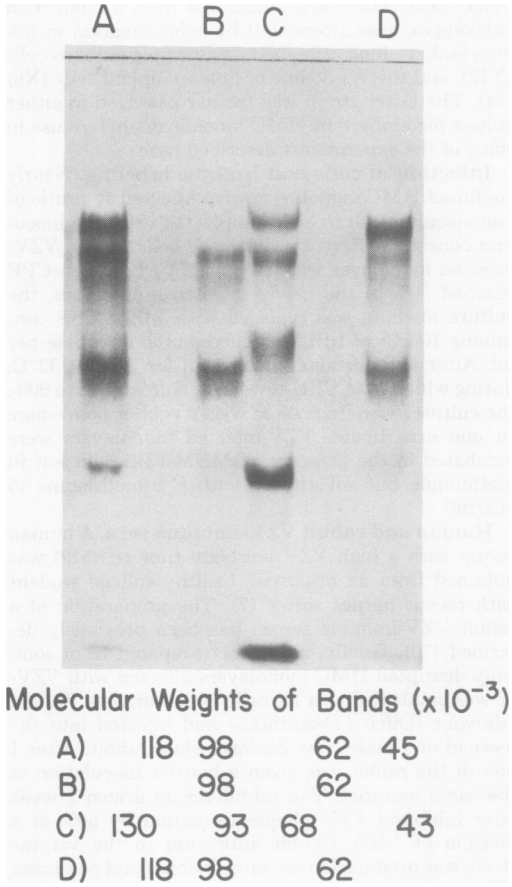

FIG. 1. *Direct immunoprecipitation of a laboratory VZV strain. Extracts of HMC cultures infected with VZV-32 in the presence of [^{3}H]glucosamine were mixed with equal volumes of human VZV-immune serum, rabbit antiserum to a VZV laboratory strain, and guinea pig antiserum to a VZV vaccine strain. The three immune precipitates (rabbit serum, lane A; guinea pig serum, lane B; and human serum, lane D) were subjected to electrophoresis in an 8% acrylamide slab gel cross-linked with MBA. Radioactive ^{14}C-labeled molecular weight standards in lane C included β-galactosidase (130,000), phosphorylase B (93,000), bovine serum albumin (68,000), and ovalbumin (43,000). The apparent molecular weights of the glycoproteins in the three immune precipitates were estimated by comparison of their mobility relative to that of the standards (21), and the results for each lane of the gel are presented in tabular format at the bottom of the figure.*

apparent molecular weights of the prominent bands are indicated in the table at the bottom of the figure. The glycoprotein profiles of the human and rabbit immune precipitates (lanes A and D, respectively) were quite similar and contained three prominent glycoproteins, which we have designated gp118, gp98, and gp62.

The profile of the guinea pig serum precipitate contained all of the major glycoproteins except gp118 (Fig. 1, lane B). A less prominent glycoprotein, gp88, not readily visualized in Fig. 1, was detectable in all three precipitates by densitometry and also after exposure of the gel to radiographic film for a total of 20 days. However, even after this increase in exposure time, gp118 was not seen in the guinea pig serum sample. Because of this discrepancy, further experiments were carried out to determine whether guinea pig antiserum lacked antibody to gp118 because of an alteration in the antigenic determinants of the vaccine strain.

Direct immunoprecipitation of VZV-Oka-infected cell extracts. HMC cultures infected with VZV-Oka were radiolabeled by the addition of tritiated sugars or [^{35}S]methionine (5 μCi/ml) to the medium for 18 to 24 h as CPE progressed from approximately 50 to >90%. Figure 2 shows the electrophoretic patterns of the VZV-Oka–human serum precipitates. The glycoprotein profile was composed of the major bands (gp118, gp98, and gp62) and closely resembled that of the VZV-32 strain after precipitation with the same antiserum (Fig. 1). An immune precipitate of an infected cell lysate labeled with [^{35}S]methionine was electrophoresed in the same slab gel to identify the polypeptides which comigrated with VZV-Oka glycoproteins (Fig. 2). These represented some of the most prominent

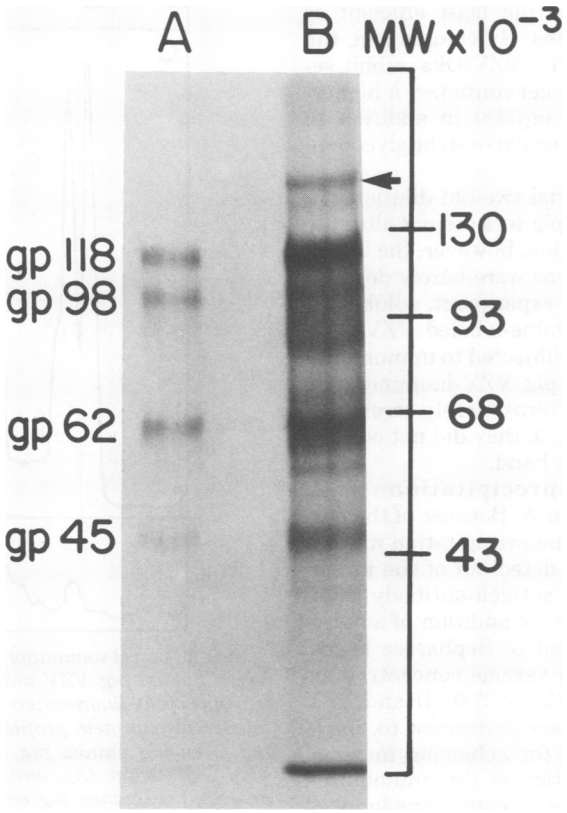

FIG. 2. *Direct immunoprecipitation of a vaccine VZV strain. One of two HMC cultures infected with strain VZV-Oka was labeled with [^{3}H]glucosamine, and the second received [^{35}S]methionine. Detergent-solubilized extracts of these cells were incubated with high-titered human VZV-immune serum. The lanes of the 8% acrylamide-MBA gel indicated by A and B contained, respectively, ^{3}H-labeled glycoproteins and ^{35}S-labeled proteins. A prominent nonglycosylated polypeptide of approximately 150,000 daltons is designated by an arrow. The ICS glycoproteins are designated on the left according to their estimated molecular weight (MW); the migration of the protein standards is diagrammatically represented on the right.*

antigens in the extract. In addition to the glycopolypeptides, another prominent but nonglycosylated protein of apparent molecular weight ~150,000 was visible in the [^{35}S]methionine sample. This high-molecular-weight polypeptide also was present in a partially purified fraction of VZV-32 (7) and probably corresponded to the major capsid polypeptide.

Infected cell extracts also were incubated with portions of rabbit antiserum to the two VZV strains. The immune precipitates were found to contain the same glycoprotein bands (fluorogram not shown; one densitometric tracing included in Fig. 3). In a similar manner, extracts were subjected to precipitation with the three VZV-immune sera produced in guinea pigs (Table 1). Figure 3 presents the densitometric tracings of the fluorogram. Each guinea pig VZV-immune serum failed to precipitate the highest-molecular-weight glycopeptide from the solubilized extract of VZV-Oka-infected cells; the antiserum to VZV-32 was the least efficient at binding ICS glycoproteins. By comparison, the glycoprotein profile of the VZV-Oka-rabbit serum from the same slab gel contained a higher-molecular-weight band (gp118) in addition to the same two lower-molecular-weight glycopeptides.

Precipitation with serial twofold dilutions (1:2 and 1:4) of the guinea pig sera did not alter the results; above a 1:4 dilution, however, the lower-molecular-weight antigens were barely detectable. In another control experiment, solubilized extracts of [^{3}H]glucosamine-labeled VZV-Oka-infected cells also were subjected to immunoprecipitation with guinea pig VZV-immune sera. The electrophoretic patterns closely resembled those represented in Fig. 3; they did not contain a high-molecular-weight band.

Indirect immunoprecipitation with staphylococcal protein A. Because of the possibility that direct immunoprecipitation was not sufficiently sensitive to detect all of the immunogenic glycoproteins, antigen-antibody complexes were precipitated by addition of staphylococcal protein A bound to Sepharose beads. Initially, titrations with various concentrations of radioactive antigen (25 to 300 μl) and VZV antibody (5 to 50 μl) were performed to determine the optimal ratio for achieving maximal precipitation after addition of the immobilized protein A (Table 2). The glycoproteins induced by VZV-32 and precipitated with rabbit VZV-immune serum and staphylococcal protein A closely resembled in molecular weight those directly precipitated with the same antiserum (Fig. 4). The two higher-molecular-weight glycopeptides as well as gp62 were present, but gp45 was

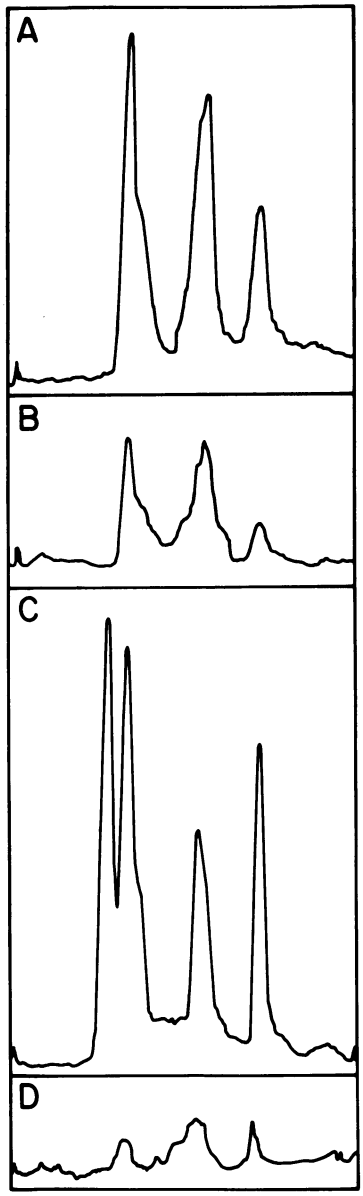

FIG. 3. *Direct immunoprecipitation with three different guinea pig VZV antisera. The densitometric tracings of the fluorograms represent the [^{3}H]fucose-labeled glycoprotein profiles after precipitation with the following guinea pig VZV-immune sera: anti-VZV-Oka strain (A), anti-VZV-Oka strain further passaged in guinea pig embryo cells (B), and anti-VZV-32 strain (D). For comparison, the densitometric tracing in (C) illustrates the electrophoretic profile after precipitation of the same virus strain with rabbit anti-Oka antiserum; it demonstrates four glycoproteins, designated gp118, gp98, gp62, and gp45 as in Fig. 1. The gel was made of 8% acrylamide with MBA cross-linkage. Migration is from left to right.*

TABLE 2. *Immunoprecipitation with various concentrations of [^{3}H]glucosamine-labeled antigen and $(NH_4)_2SO_4$-precipitated HMC-adsorbed antiserum*

Lane[a]	Labeled antigen (μl)	Antibody (μl)	Radioactivity precipitated (cpm)	Radioactivity added to well (cpm)
A	100	25	37,440	18,720
B	200	25	59,860	29,930
C	100	10	31,740	15,870
D	100	25	44,180	28,717

[a] Refers to slab gel pictured in Fig. 4.

not well visualized. The recurrent problem of obtaining sufficiently radioactive antigen was at least partially overcome by using tritiated glucosamine of high specific activity (38 Ci/mmol) or fucose which had been doubly substituted to achieve an even higher activity (56 Ci/mmol). In general, 15 to 20% of the total radioactivity present in the original infected cell extract was recovered after one immunoprecipitation. A second addition of protein A to the supernatant did not precipitate more antigen.

When the indirect immune precipitates with guinea pig VZV antisera were compared with

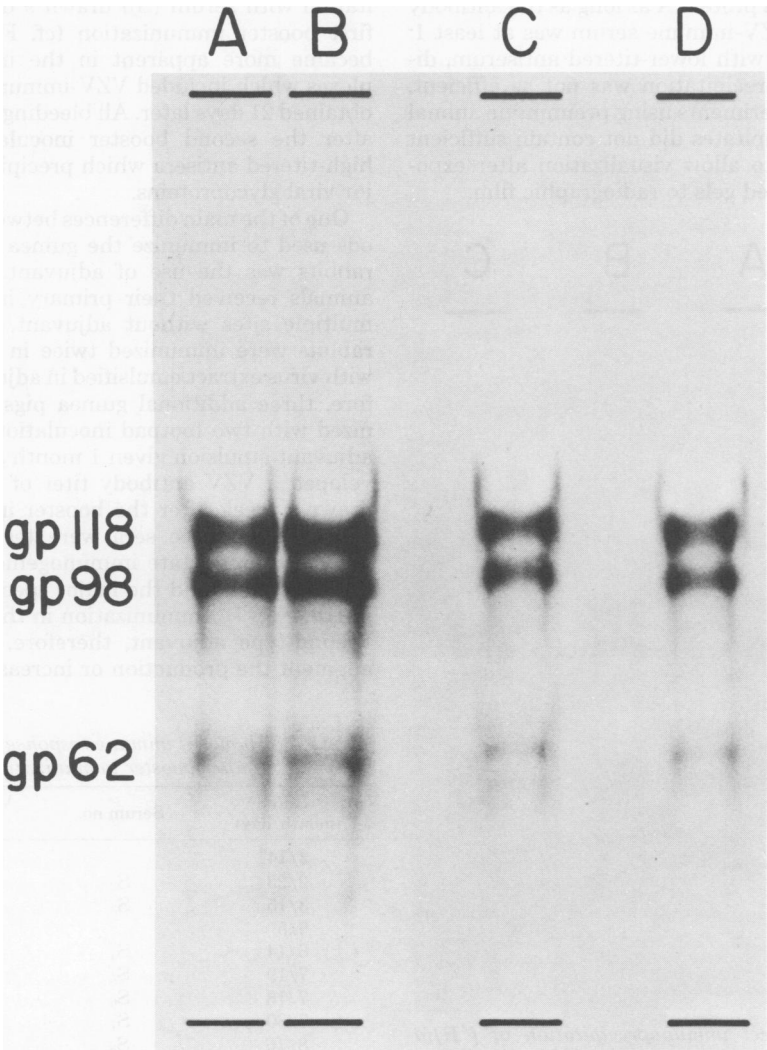

FIG. 4. *Indirect immunoprecipitation of a detergent-solubilized extract of [^{3}H]glucosamine-labeled VZV-infected cells with rabbit VZV-immune serum. The amount of radioactive antigen applied to wells A to D is given in Table 2. Electrophoresis was performed in an 8% acrylamide slab gel cross-linked with DATD.*

those of rabbit and human antisera, the electrophoretic patterns were similar to the fluorograms seen in Fig. 1 to 3; i.e., the latter two sera precipitated the major VZV glycoprotein antigens, whereas the lanes containing the guinea pig serum-precipitate appeared to lack a higher-molecular-weight antigen (Fig. 5). Enhancement of precipitation by protein A, however, did on occasion allow visualization of a minor band in the region corresponding to gp118, especially if the wells were grossly overloaded.

When the electrophoretic profiles after direct and indirect immunoprecipitations were compared, it was apparent that the sensitivity was not noticeably increased by the addition of staphylococcal protein A as long as the antibody titer of the VZV-immune serum was at least 1:128; however, with lower-titered antiserum, direct immunoprecipitation was not as efficient. In control experiments using preimmune animal sera, the precipitates did not contain sufficient radioactivity to allow visualization after exposure of the dried gels to radiographic film.

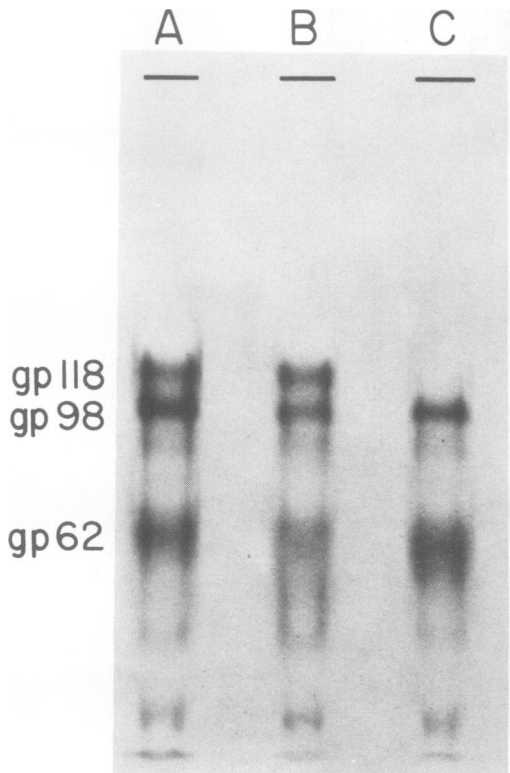

FIG. 5. *Indirect immunoprecipitation of [^{3}H]fucose-labeled glycoproteins with rabbit (A), human (B), and guinea pig ii (C) VZV antisera. The precipitates were subjected to electrophoresis in an 8% acrylamide (DATD cross-linked) slab gel.*

Maturation of the guinea pig VZV antibody response. It is recognized that hyperimmunization of laboratory animals produces a broadened although less specific antibody response (11, 15). To determine whether the differences we observed between rabbit and guinea pig antisera were the result of heterogeneous responses to VZV immunogens, we continued to bleed and reimmunize guinea pig ii (Table 3). When the more convalescent antisera were used for antigen precipitation, the glycoprotein profiles began to closely resemble those of hyperimmune rabbit or human zoster antisera (Fig. 6). The highest-molecular-weight glycopeptide, gp118, which was barely detectable after precipitation with serum (S_2) drawn 9 days after the first booster immunization (cf. Fig. 1 and 5), became more apparent in the immune complexes which included VZV-immune serum (S_3) obtained 21 days later. All bleedings 1 to 6 weeks after the second booster inoculation yielded high-titered antisera which precipitated all major viral glycoproteins.

One of the main differences between the methods used to immunize the guinea pigs and the rabbits was the use of adjuvant. The former animals received their primary inoculation at multiple sites without adjuvant, whereas the rabbits were immunized twice in the footpads with virus extract emulsified in adjuvant. Therefore, three additional guinea pigs were immunized with two footpad inoculations of a virus-adjuvant emulsion given 1 month apart. All developed a VZV antibody titer of 1:512 in sera drawn 1 week after the booster immunization. When these three sera were tested for their ability to precipitate immunogenic VZV glycoproteins, all bound the major species, including gp118 (Fig. 7). Immunization in the presence of Freund-type adjuvant, therefore, appeared to augment the production or increase the avidity

TABLE 3. *Humoral immune response of guinea pig ii after booster immunizations*

Date (month/day)	Serum no.	VZV antibody titer
2/14[a]		
2/23	S_2	1:256
3/15	S_3	1:256
6/5[a]		
6/14	S_4	1:256
7/19	S_5	1:256
7/18	S_6	1:256
7/30	S_7	1:128
8/16	S_8	1:64

[a] Booster immunizations were given 6 and 10 months, respectively, after the primary inoculation with VZV-Oka.

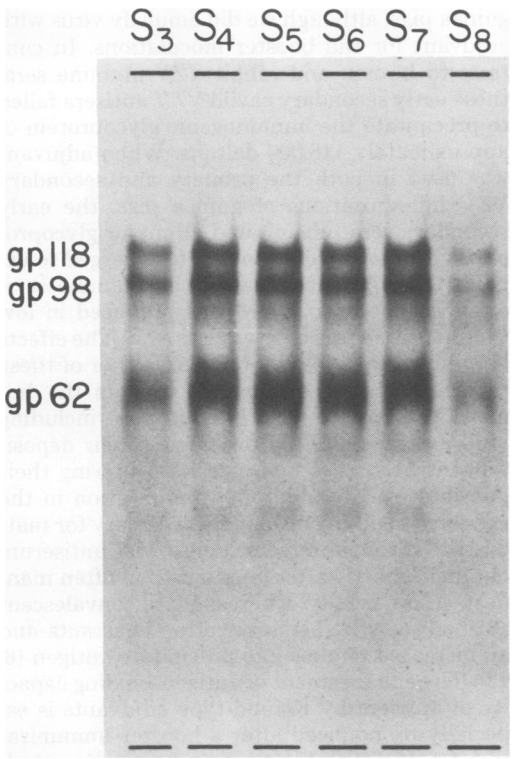

FIG. 6. *Indirect immunoprecipitation of [^{3}H]fucose-labeled VZV antigens with serial guinea pig VZV antisera drawn up to 6 months after booster immunization (see Table 3). Electrophoresis was performed in a DATD-cross-linked 8% acrylamide slab gel.*

of early secondary antiviral antibody for the high-molecular-weight glycoprotein determinant.

DISCUSSION

As part of an evaluation of the immunogenicity of VZV extracts in laboratory animals, we immunized guinea pigs and rabbits with either a laboratory (VZV-32) or a vaccine (VZV-Oka) strain. When the animal VZV-immune sera, as well as a human zoster serum, were incubated with detergent-solubilized extracts of either VZV-32- or VZV-Oka-infected cells cultures, similar amounts of a glycosylated polypeptide of molecular weight 62,000 and two higher-molecular-weight species (gp98 and gp118) were precipitated. In addition, both strains possessed a prominent high-molecular-weight (~150,000) nonglycosylated polypeptide which appears to be common to all herpesviruses (14). Thus, both a low-passage laboratory strain and the vaccine virus exhibited the same major determinants, which corresponded in molecular weight to ICS proteins previously elucidated in VZV-infected HMC cultures (7). Two other ICS glycoproteins, gp88 and gp45, were not consistently observed in the immune precipitates. Pending identification of the virion-specific polypeptides, we have

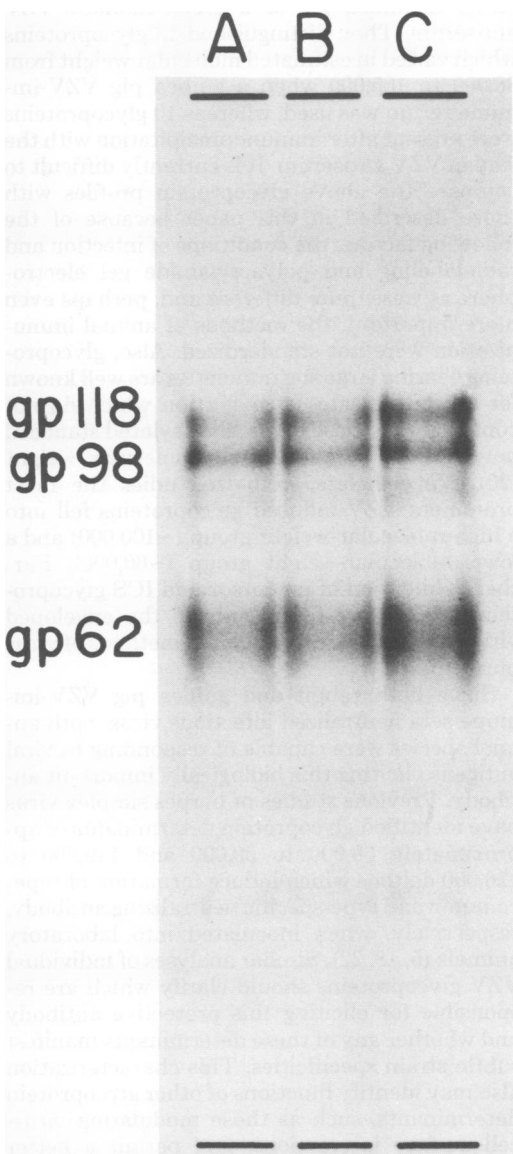

FIG. 7. *Indirect immunoprecipitation with three additional guinea pig VZV antisera. Each of three guinea pigs was immunized twice with VZV cell extracts emulsified in complete Freund adjuvant and bled 1 week after the booster inoculation. The [^{3}H]fucose-labeled glycopeptides precipitated by the three high-titered VZV-immune sera were analyzed by electrophoresis in a DATD-cross-linked 10% acrylamide slab gel.*

not attempted to further define several (at least eight) less prominent nonglycosylated determinants which were presented in Fig. 2.

Other investigators (3) also have described electrophoretic patterns after precipitation of radiolabeled VZV-induced glycopeptides with either a guinea pig or a green monkey VZV antiserum. They distinguished 13 glycoproteins which varied in estimated molecular weight from 38,000 to 105,000 when a guinea pig VZV-immune serum was used, whereas 10 glycoproteins were present after immunoprecipitation with the simian VZV antiserum. It is currently difficult to compare the above glycoprotein profiles with those described in this paper because of the following factors: the conditions of infection and radiolabeling and polyacrylamide gel electrophoresis were quite different and, perhaps even more important, the methods of animal immunization were not standardized. Also, glycoproteins bearing large sugar moieties are well known for exhibiting rates of migration which do not conform with those of nonglycosylated standard polypeptides of comparable molecular weight (20). Nevertheless, in both studies the most prominent VZV-induced glycoproteins fell into a high-molecular-weight group (~100,000) and a lower-molecular-weight group (~60,000). Further delineation of precursor and ICS glycoproteins from those found only in the enveloped virion will require more defined methods of virus purification.

Since both rabbit and guinea pig VZV-immune sera neutralized infectious virus, both animal species were capable of responding to viral antigens eliciting this biologically important antibody. Previous studies of herpes simplex virus have identified glycoprotein determinants of approximately 58,000 to 59,000 and 119,000 to 126,000 daltons which induce formation of type-common and type-specific neutralizing antibody, respectively, when inoculated into laboratory animals (5, 18, 22). Similar analyses of individual VZV glycoproteins should clarify which are responsible for eliciting this protective antibody and whether any of these determinants manifest subtle strain specificities. This characterization also may identify functions of other glycoprotein determinants, such as those modulating virus-cell surface interactions, and permit a better understanding of the biology of both the cell-associated in vitro VZV infection and the recently described in vivo infection of guinea pigs (17).

A second important observation of this paper relates to the maturation of the immune response in guinea pigs immunized with VZV extracts. In our initial experiments, we did not use adjuvant in the primary immunizations of guinea pigs, although we did emulsify virus with adjuvant for the booster inoculations. In contrast to human and rabbit VZV-immune sera, these early secondary caviid VZV antisera failed to precipitate the immunogenic glycoprotein of approximately 118,000 daltons. When adjuvant was used in both the primary and secondary VZV immunizations of guinea pigs, the early secondary sera precipitated all major glycoproteins. Two possible explanations emerged to explain the differences among the immune sera: either antibody to gp118 was produced in low titer in guinea pigs or it was less avid. The effects of adjuvant action may relate to either of these two possibilities. Adjuvant augments the humoral response to most immunogens, including viral antigens (25), by prolonging their deposition in the tissues and, possibly, altering their presentation. In addition, immunization in the presence of adjuvant may be necessary for maturation of the immune response; viz., antiserum obtained shortly after immunization often manifests a low avidity, whereas more convalescent antibody has higher association constants and an increased tendency to precipitate antigen (6, 23). The enhancement of antigen-binding capacity of antisera by Freund-type adjuvants is especially pronounced after a booster immunization (1). Incidental to these immunization studies in guinea pigs, we have not noted a difference in immunogenic potential between low-passage laboratory and vaccine VZV strains (cf. reference 26).

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