Immunity in strain 2 guinea-pigs inoculated with vaccinia virus recombinants expressing varicella-zoster virus glycoproteins I, IV, V or the protein product of the immediate early gene 62


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The immunogenicity of specific varicella-zoster virus (VZV) proteins, with emphasis upon cell-mediated immune responses, was evaluated by immunizing strain 2 guinea-pigs with vaccinia virus recombinants that express gpI (vac-gpI), gpIV (vac-gpIV) and gpV (vac-gpV) or the IE-62 protein (vac-IE-62). Vac-gpI elicited the highest initial mean T cell proliferation response [stimulation index (S.I.) 3.8 ± 0.9 S.E.M.] whereas inoculation with vac-gpV produced the lowest primary T cell response (S.I. 2.5 ± 1.1 S.E.M.). T cell proliferation was detected for a shorter period after immunization with vac-gpV compared to vac-gpI, vac-gpIV or vac-IE-62. A comparison of the immunogenicity of vac-gpI and vac-IE-62 with the same proteins prepared by immunoaffinity purification showed that immunization with these proteins in either form elicited virus-specific IgG antibodies and T cell recognition. The presence or absence of IgG antibodies to the IE-62 protein was used to assess protection against challenge with guinea-pig cell-adapted infectious VZV in animals that had been inoculated with vac-gpI, vac-gpIV or vac-gpV. Immunization with vac-gpI and vac-gpIV restricted VZV replication but all animals given vac-gpV developed antibodies to IE-62 after challenge with infectious VZV. Priming of the T lymphocyte response was observed in all animals immunized with VZV–vaccinia virus recombinants after subsequent exposure to infectious VZV. These experiments with VZV vac-gpI, vac-gpIV and vac-gpV in guinea-pigs suggest variability in the capacity of herpesviral glycoproteins to elicit cell-mediated immunity in vivo. Induction of virus-specific immunity using IE-62 means that this major tegument protein of VZV could be a useful component for vaccine development.

Introduction

Varicella-zoster virus (VZV), which is an alphaherpesvirus, is the aetiological agent of varicella and also causes herpes zoster, a reactivation of latent virus involving the dermatomal distribution of one or more sensory neurons. Investigations of the pathogenesis of infection and the host response to VZV have been complicated by the difficulty of infecting other species with this human herpesvirus. Simian varicella virus has some antigenic similarities to VZV, but infection caused by simian varicella virus tends to be much more severe than that caused by VZV in the healthy human host (Ayres, 1971).

Human VZV is immunogenic in some non-human primates but the animals do not develop the usual clinical signs of varicella (Felsenfeld & Schmidt, 1979). Although the manifestations of infection are also limited, the guinea-pig provides a less costly animal model for studies of VZV infection. Early observations showed that strain 2 guinea-pigs developed virus-specific antibodies in high titre following inoculation with VZV that had been adapted by passage in guinea-pig embryo cells in vitro; infectious virus was also present in the nasopharynx and blood of some of these animals (Myers et al., 1980, 1985). Animal-to-animal transmission occurred regardless of the original route of VZV inoculation, suggesting that VZV infection of the guinea-pig became generalized (Myers et al., 1980; Matsunaga et al., 1982). A mononuclear cell-associated viraemia can be demon-
strated by in situ hybridization in guinea-pigs inoculated with VZV, with the frequency of infected cells being equivalent to that observed in healthy human subjects with varicella (Koropchak et al., 1989). Recently, the polymerase chain reaction was used to detect the virus in skin lesions produced by infecting euthemic, hairless Hartley guinea-pigs with VZV (Myers et al., 1991).

Since cell-mediated immunity is critical in the human host response to VZV, the fact that VZV-specific T cell proliferation and delayed hypersensitivity responses to VZV antigens are elicited by VZV infection makes the guinea-pig model especially useful (Matsunaga et al., 1982; Arvin et al., 1986a, 1987). T lymphocyte-mediated cytotoxicity against histocompatibility-matched, VZV-infected target cells can be demonstrated by infecting inbred guinea-pig strains with VZV (Hayward et al., 1991). VZV infection also induces antibodies with neutralizing activity and antibodies that immunoprecipitate the same VZV-infected cell proteins as human immune sera (Grose & Friedrichs, 1982). With regard to the viral protein specificity of the immune response, immunoaffinity-purified glycoprotein I (gpI) and an immediate early (IE) protein (IE-62) were found to be immunogenic in strain 2 guinea-pigs (Arvin et al., 1987). These proteins are also targets of persistent immunity to VZV in human subjects following wild-type virus infection and after immunization with the Oka/Merck varicella vaccine (Arvin et al., 1986b, 1991; Diaz et al., 1988). The immunogenicity of the IE-62 protein is of particular interest because it has now been characterized as a regulatory protein that also constitutes a major component of the virion tegument (Kinchingon et al., 1991). In contrast, the herpes simplex virus (HSV) homologue ICP4 is a minor tegument protein (Yao & Courtney, 1989).

The purpose of the present work was to use the guinea-pig model to evaluate the host response, particularly cell-mediated immunity, elicited by specific VZV proteins in animals immunized with vaccinia virus recombinants that express gpI, gpIV and gpV, or the IE-62 protein. VZV gpI, gpIV and gpV are homologous to HSV gE, gI and gC respectively, and appear to share at least some of the properties of their HSV counterparts. Evidence from a number of sources has shown that all three VZV glycoproteins are neutralizing antigens, either in the presence or absence of complement, when tested in virus growth assays in vitro (reviewed in Gelb, 1990). However, although VZV glycoproteins and IE-62 protein are known targets of the host response, the role of these proteins or of any other VZV proteins in protection against VZV infection has not been assessed in animal models. In these experiments, the host response to VZV gpI and IE-62 expressed in vaccinia virus was compared with that elicited by the corresponding immunoaffinity-purified proteins and the protective effect of prior immunization with the VZV–vaccinia virus recombinants expressing viral glycoproteins upon challenge with infectious VZV was determined. Since the detection of infectious VZV is not reliably reproducible in the guinea-pig model, protection against VZV replication was assessed by testing challenged animals for the production of antibodies to the IE-62 protein.

### Methods

**Vaccinia virus recombinants expressing VZV proteins.** Vaccinia virus recombinants were constructed using the vector pSC11 (Chakrabarti et al., 1985). Procedures for constructing vaccinia virus recombinants expressing VZV gpI (vac-gpI), gpV (vac-gpV) and IE-62 (vac-IE-62) have been described (Moss & Flexner, 1987; Kinchingon et al., 1990, 1991).

A new VZV–vaccinia virus recombinant, vac-gpIV, was constructed and used in the present experiments. VZV gpIV was obtained by cutting the BamH I K fragment of VZV strain Scott with AccI to give a 1256 bp fragment containing the VZV gpIV open reading frame (ORF) 67 (nucleotides 114496 to 115558) (Davison & Scott, 1986). These fragments were cloned into pSC11 at the Sma I site, in the correct orientation, and the viruses were recombined into vaccinia virus strain WR, as previously described (Chakrabarti et al., 1985). The distance, on the 5′ side, between the start of VZV sequences and the VZV gpIV ATG is 26 bp, and there are no ATG codons in this sequence. As a comparison, the analogous distances for our gpI, gpV and IE–62–vaccinia virus recombinants are 90, 2 and 93 bp. Screening for poxvirus early transcription termination motifs (TTTTTTNT or TTTTTTTNT) within VZV sequences in the recombinants revealed one each in gpI and gpV (positions 117236 to 117243 and 20260 to 20266 respectively) and none in gpIV or IE–62. Nevertheless, expression from all four VZV ORFs in the vaccinia virus recombinants in vitro was substantial (e.g. Fig. 1) and approximately equivalent, with no one recombinant appearing to be more or less capable of producing apparently authentic protein than the others. No evidence that truncated forms of any of these VZV proteins are made has been obtained. Expression of VZV gpIV by vac-gpIV was verified by preparing antisera against vac-gpIV in rabbits by a method previously described (Kinchingon et al., 1990); these antisera were then used to precipitate proteins from VZV-infected cell extracts. VZV-infected cells were radiolabelled with [35S]methionine and [14C]glucosamine, washed in cold PBS, scraped off and pelleted. Cell pellets were treated with 1% NP40 in PBS at 4 °C. After vortexing, nuclei and cellular debris were removed by ultracentrifugation, and the cytoplasmic extracts were used in immunoprecipitation experiments. Immunoprecipitation was performed by mixing antisera collected from rabbits infected with vac-gpIV with the extract at a ratio of 1:10; after 24 h, the precipitates were collected using Protein A-Sepharose. GpIV expression was also tested by infecting human foreskin fibroblast (HFF) cells with vac-gpIV and performing immunoblots with hyperimmune serum from an individual convalescing from herpes zoster.

In the studies described here, the vaccinia virus vSC8 served as a control recombinant; it contains p11, β-galactosidase, p7.5 and vaccinia virus thymidine kinase sequences but has no foreign viral DNA insert (Chakrabarti et al., 1985). This recombinant, the pSC11 vector and the WR strain of vaccinia virus were generously provided by Dr Bernard Moss, LVD, NIAID, Bethesda, Md., U.S.A.
Preparation of guinea-pig-adapted infectious VZV. Foetal guinea-pig cell lines were established from guinea-pig embryos of 1-0 to 1-5 cm and were viable for up to six passages; cells were used to support growth of guinea-pig cell-adapted VZV (Edmond et al., 1981). After its original isolation in human melanoma cells, a clinical VZV isolate, designated strain Chase, was passaged five times in HFF cells. Infected fibroblasts were then inoculated onto guinea-pig embryo cells, using VZV-infected cells to inoculate the fresh cell monolayer at a ratio of 1:4; infected guinea-pig embryo cells were then further sub-passaged 10 times onto guinea-pig embryo cell monolayers. This guinea-pig cell-adapted VZV stock was stored as infected cells at −70 °C or in liquid N₂, and passaged once more in guinea-pig embryo cells immediately before the inoculation of animals.

Preparation of immunoaﬃnity-puriﬁed VZV proteins. VZV proteins were isolated from infected cells using monoclonal antibodies (MAbs) to VZV gpI (90K/58K Mr, complex) or to the IE-62 protein (175K to 180K) (Arvin et al., 1986b). The MAbs were coupled to cyanogen bromide-activated Sepharose 4B and incubated with a solubilized extract of human melanoma cells or of Vero cells infected with vac-gpI or vac-IE-62. Bound proteins were eluted with 3 M potassium thiocyanate pH 7.5, followed by centrifugation and vacuum concentration of the eluate. An uninfected cell control preparation was made in parallel. Each preparation was examined by SDS-PAGE and by immunoblot reactivity with polyclonal antibodies and MAbs. As described previously, the conditions required for immunoaﬃnity separation and for immunoblot analysis disrupt the 170K IE-62 protein, yielding multiple lower Mr bands that retain antigenic reactivity with protein-specific antibodies (Arvin et al., 1986b).

Animals and inoculation procedures. Weanling strain 2 guinea-pigs were obtained from the Charles River Caviary and the National Cancer Institute, Bethesda, Md., U.S.A. The protein antigens were diluted in PBS and given as two doses (50 µg protein/dose) with a 1 week interval between injections; adjuvant was not used. The VZV–vaccinia virus recombinants, using approximately 10⁵ p.f.u./dose, were inoculated onto a scarified skin area just below the thorax and produced visible cutaneous lesions within 3 days; a second inoculation was given 3 weeks later. Control animals were inoculated with vaccinia virus vSC8. Experiments were carried out with cohorts of eight animals for each immunogen. Animals given guinea-pig-adapted infectious VZV were inoculated with 1 × 10⁶ VZV-infected guinea-pig cells subcutaneously as a single dose of 0-25 to 0-45 ml. Challenge experiments in animals initially given VZV–vaccinia virus recombinants were done with cohorts of four animals. Blood samples were obtained by cardiac puncture using general anaesthesia induced with xylazine (5 mg/kg) and ketamine (44 mg/kg).

Immunoblot assay. Serum IgG antibodies reactive with specific VZV proteins were detected by immunoblotting. Immunoaﬃnity-puriﬁed VZV proteins prepared with the anti-gpI and anti-IE-62 MAbs and control preparations were separated by SDS-PAGE in a 9% acrylamide gel crosslinked with methylene bisacrylamide followed by electrophoretic transfer to nitrocellulose with a Tramabol chamber (Bio-Rad). The strips were blocked with 10% (w/v) Carnation instant non-fat dried milk containing 0-15 m-NaCl, 50 mM-Tris–HCl pH 7-6, 3 mM-sodium azide, 5 mM-EDTA and 0-05% (v/v) Tween 20, for 1 to 1-5 h. The nitrocellulose strips were then incubated with the test sera at a dilution of 1:10 in the same buffer for 1-5 to 2-0 h. Bound IgG was detected by immunoperoxidase staining using biotinylated goat antibody to guinea-pig IgG (Vectastain ABC kit, Vector Laboratories). The Mr of visible bands was estimated using reference standards. Preimmune sera and sera from animals inoculated with the vaccinia virus control or with uninfected guinea-pig embryo cells were included as controls.

Cell-mediated immunity assay. VZV-specific T lymphocyte proliferation was determined by incubating peripheral blood mononuclear cells, obtained by cardiac puncture and separated with Ficoll–Hypaque, with VZV antigen or an uninfected cell control in 96-well microtitre plates (Arvin et al., 1987). The cells were cultured at a concentration of 3 × 10⁴ cells/0-2 ml per well in RPMI medium with 10% guinea-pig serum, 2-mercaptoethanol and 5% NaHCO₃. Duplicate wells were incubated with antigen or control in ratios of 1:4, 1:16 and 1:64. After 6 days, the cells were pulse-labelled with [³H]thymidine. On day 7, the cells were harvested onto glass filter paper and counted in a liquid scintillation counter. The stimulation index (S.I.) was calculated as the ratio of mean c.p.m. in antigen-stimulated wells to the mean c.p.m. in duplicate control wells, with an S.I. > 2.0 being considered positive (Arvin et al., 1987). Each assay included positive control wells stimulated with phytohaemagglutinin.

Statistical analysis. Mean S.I.s were calculated and compared using Student’s t-test; the software package Statview II (Apple Computer) was used for data entry and analysis.

Results

Expression of VZV gpIV by the vaccinia virus recombinant, vac-gpIV

When HFF cells infected with vac-gpIV were tested for VZV gpIV expression by immunoblot assay with hyperimmune serum from an individual convalescing from herpes zoster, a weak reactivity with 60K, 50K and 45K species was found (data not shown). To confirm expression of gpIV by the recombinant, rabbit antiserum was prepared and was used to precipitate protein extracts from VZV-infected radiolabelled cells. Fig. 1 illustrates the immunoprecipitation of radioactively labelled extracts from VZV-infected cells using rabbit antiserum against vac-gpIV. Lane 1 shows immunoprecipitated proteins labelled for 24 h with glucosamine, indicating a major sugar-labelled species at about 60K. Lane 2 contains proteins labelled in a pulse for 15 min with [³5S]methionine and reveals a major species at about 50K and a minor 45K band. If the pulse-labelled material is chased with unlabelled methionine for 4 h and then immunoprecipitated (lanes 3 and 4), the 60K band become prominent and the 50K band is substantially diminished. Since the gpIV ORF encodes a product of about 40K (Davison & Scott, 1986), we interpret these data to show that the 60K form is the mature, glycosylated gpIV and the smaller species are less-processed forms. These results fit with the earlier description of VZV gpIV, obtained using a different approach (Davison et al., 1985). In control experiments, sera from rabbits immunized with a vaccinia virus recombinant carrying the VZV gpV gene were tested, along with preimmune sera, in the above assay. The highest Mr band seen in Fig. 1 (most obvious in lanes 3 and 4) appeared in both of these blots. We believe that
VZV-specific T lymphocyte proliferation in guinea-pigs immunized with vac-gpI, vac-gpIV, vac-gpV or vac-IE-62 compared to that in guinea-pigs inoculated with guinea-pig cell-adapted infectious VZV

As shown in Table 1, all of the VZV–vaccinia virus recombinants elicited T lymphocyte proliferative responses against VZV antigen. Vac-gpI induced the highest mean T cell proliferation at 3 weeks after the second dose (S.I. 3.8 ± 0.9 S.E.M.) and was the only VZV–vaccinia virus recombinant to which a proliferative response was still detected at 15 to 18 weeks (S.I. 2.1 ± 0.8 S.E.M.). Inoculation with vac-gpV produced the lowest initial lymphoproliferative response at 3 weeks (S.I. 2.5 ± 1.1 S.E.M.) and T cell proliferation persisted for a shorter interval after immunization with vac-gpV compared to vac-gpI, vac-gpIV or vac-IE-62. Animals inoculated with infectious guinea-pig cell-adapted VZV had higher T lymphocyte proliferation which was detectable for a longer time than animals given any of the VZV–vaccinia virus recombinants.

Comparison of VZV immunity elicited by gpI or IE-62 presented by vaccinia virus recombinants and immunization with immunoaffinity-purified gpI or IE-62

The development of humoral immunity was evaluated by immunoblotting in guinea-pigs immunized with vac-gpI or vac-IE-62. IgG antibodies to VZV proteins were detected in all animals tested 3 weeks after a second inoculation with vac-gpI (four of four) or vac-IE-62 (four of four) vaccinia virus recombinants, as also observed in animals given immunoaffinity-purified gpI or IE-62. None of the four animals given the control vaccinia virus recombinant developed VZV antibodies (data not shown).

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Table 1. The persistence of VZV-specific T lymphocyte proliferation in guinea-pigs inoculated with vac-gpI, vac-gpIV, vac-gpV or vac-IE-62 compared to that in guinea-pigs inoculated with guinea-pig cell-adapted infectious VZV

<table>
<thead>
<tr>
<th>VZV–vaccinia virus recombinant</th>
<th>Interval after immunization</th>
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<tr>
<td>Vac-gpI*</td>
<td>3 weeks</td>
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<tr>
<td>Vac-gpIV</td>
<td>6 to 12 weeks</td>
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<tr>
<td>Vac-gpV</td>
<td>15 to 18 weeks</td>
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<tr>
<td>Vac-IE-62</td>
<td>3 weeks</td>
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<tr>
<td>Vac-c</td>
<td>6 to 12 weeks</td>
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<tr>
<td>VZV</td>
<td>15 to 18 weeks</td>
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* Animals given VZV–vaccinia virus recombinants were tested 3 weeks following the second inoculation.
† S.I. ± S.E.M.
Immunity to VZV glycoproteins

The acquisition of T lymphocyte proliferation responses was assessed 3 weeks after the second inoculation of vac-gpI or vac-IE-62 and compared to cell-mediated immunity in animals tested 3 weeks after the second injection of immunoaffinity-purified gpI or IE-62 protein (Fig. 2). All of the animals in each cohort had detectable cellular immunity (S.I. > 2.0) at this time interval, regardless of whether they were immunized with vac-gpI or vac-IE-62, or with the corresponding immunoaffinity-purified proteins. The mean S.I. for guinea-pigs given vac-gpI was 3.8 ± 0.9 S.E.M. compared to a mean S.I. of 5.3 ± 3.1 S.E.M. for animals given immunoaffinity-purified gpI; this difference was not statistically significant (P, 0.64, Student's t-test). The mean S.I. in animals inoculated with vac-IE-62 was 3.6 ± 0.8 S.E.M. In comparison, the mean S.I. in the cohort given purified IE-62 was 7.8 ± 2.8 S.E.M. However, this difference was also not statistically significant (P, 0.18, Student's t-test). When tested at 12 and 18 weeks, all animals inoculated with immunoaffinity-purified protein preparations of gpI and IE-62 had S.I.s < 2.0.

Effect of immunization with vac-gpI, vac-gpIV or vac-gpV on infection with guinea-pig cell-adapted VZV

The production of IgG antibodies directed against IE-62 was used as a marker to assess the capacity of guinea-pig cell-adapted infectious VZV to replicate in strain 2 guinea-pigs that had been inoculated with vac-gpI, vac-gpIV or vac-gpV. The challenge with infectious VZV was carried out 3 weeks after the second inoculation of each VZV-vaccinia virus recombinant. Serum samples were tested by immunoblotting against an IE-62 antigen preparation that had been made by immunoaffinity purification from Vero cells infected with vac-IE-62. As illustrated by representative immunoblots in Fig. 3, antibodies to IE-62 were not detected in any of the animals immunized with vac-gpI (nought of four) or vac-gpIV (nought of four). In contrast, all of the animals given vac-gpV (four of four) had antibodies to IE-62 in serum obtained 3 weeks after challenge with infectious VZV. Antibodies to IE-62 were present in serum from control animals challenged with infectious VZV (three of three).

Cell-mediated immunity in animals given vac-gpI, vac-gpIV, vac-gpV or vac-IE-62 followed by inoculation with guinea-pig cell-adapted infectious VZV

VZV-specific T lymphocyte proliferation was evaluated in animals that had been immunized with each of the...
permitted the analysis of the host response to this viral protein, gpI, as part of a comparative evaluation of the immunogenicity of vaccinia virus recombinants expressing VZV gpl, gpV and IE-62 protein in strain 2 guinea-pigs. The proteins made by vac-gpIV were similar to the glycosylated 45K to 60K products of the gpIV gene that have been identified using ORF 67-specific antipeptide sera or MAbs (Davison et al., 1985; Vafai et al., 1988). The heterogeneous polypeptides detected in cells infected with vac-gpIV are likely to be related through precursor/product processing, due to the addition and processing of carbohydrate. The 60K form of gpIV was the most abundantly glycosylated species and is probably the terminal form since it predominated in pulse–chase experiments.

The vaccinia virus strains that produced VZV glycoproteins gpI, gpV or gp induced virus-specific immunity in the guinea-pig model, as has been observed in animal studies using vaccinia virus recombinants that express HSV glycoproteins as immunogens (Cremer et al., 1985; Wachsman et al., 1987; Willey et al., 1988; Weir et al., 1989; Sekulovich et al., 1990). The herpesviral glycoproteins have been considered good candidate antigens for vaccine development because many carry epitopes for neutralizing antibodies (Friedrichs & Grose, 1984; Keller et al., 1984). However, our experiments with VZV vac-gpI, vac-gpIV and vac-gpV in guinea-pigs indicate some variability in the capacity of representatives of this class of viral proteins to elicit T cell proliferative responses in vivo. T cell recognition of VZV gpI was detected longer than responses to gpIV or gpV, and gpIV induced more prolonged cellular immunity than gpV. Although herpesviral glycoproteins are clearly immunogenic, the fact that T lymphocytes recognize short amino acid sequences of processed proteins, presented in the context of major histocompatibility antigens, means that other classes of viral proteins can be important immunogens. Among the herpesviruses, the major immediate early protein of cytomegalovirus was the first non-glycoprotein to be identified as an immunodominant T cell target (Gönczöl et al., 1986). The present experiments in strain 2 guinea-pigs demonstrated the immunogenicity of the VZV regulatory and tegument protein, IE-62, when expressed in vaccinia virus. The HSV VP16, although not homologous to IE-62, is an HSV tegument protein that also elicited immunity in murine and guinea-pig models when expressed in a vaccinia virus recombinant (Sekulovich et al., 1990). Given our previous observations that VZV IE-62 protein is a target of persistent helper and cytotoxic T cell immunity in the human host, the finding that virus-specific cellular immunity can be induced using IE-62 means that this major tegument protein of VZV could be a useful component for vaccine development.

VZV–vaccinia virus recombinants and then challenged with infectious VZV 3 weeks after the second inoculation of the recombinant. As shown in Fig. 4, the exposure to infectious VZV resulted in a marked increase in T lymphocyte proliferation in all of the animals from each cohort. The maximum S.I. was observed in animals tested at 6 to 12 weeks after the second vaccinia virus inoculation, which represented an interval of 3 to 9 weeks following VZV challenge, among those animals given vac-gpIV, vac-gpV or vac-IE-62, and at 15 to 18 weeks for those given vac-gpI. The mean peak S.I. was 10-6 ± 3-1 S.E.M., for the cohort initially immunized with vac-gpI, 14-1 ± 4-1 S.E.M. for animals given vac-gpIV, 11-2 ± 4-5 S.E.M. for those given vac-gpV and 10-1 ± 2-7 S.E.M. for those given vac-IE-62. The mean peak S.I. for animals immunized with control recombinant vaccinia virus (vac-c) then challenged with VZV was 1-2 ± 0-3 S.E.M.

**Discussion**

The construction of a new vaccinia virus recombinant that expressed proteins derived from ORF 67 of VZV

![Fig. 4. VZV-specific T lymphocyte proliferation in immunized strain 2 guinea-pigs after challenge with infectious VZV. VZV-specific T lymphocyte proliferation responses, expressed as the mean S.I. ± S.E.M., in animals immunized with vac-gpI (●), vac-gpIV (○), vac-gpV (▲) or vac-IE-62 (△) followed by inoculation with guinea-pig cell-adapted infectious VZV. The animals were tested before and at intervals of 6 to 12 weeks and 15 to 18 weeks after challenge with infectious VZV. The mean peak S.I. for animals immunized with the vaccinia virus control recombinant (vac-c) then challenged with VZV was 1-2 ± 0-3 S.E.M.](image-url)
In contrast to immunization with vac-gpI or vac-gpIV, guinea-pigs given vac-gpV were not protected from VZV replication, as demonstrated by the production of antibodies to the internal virion IE-62 protein following challenge with guinea-pig cell-adapted VZV. Antibody production to this protein proved to be a useful marker of VZV infection in the guinea-pig, particularly because the limited phase of active virus replication and its unpredictable timing after inoculation makes it very difficult to document VZV infection of these animals by viral culture. In similar experiments, the production of antibodies to HSV ICP-35 protein was used to demonstrate asymptomatic infection in guinea-pigs challenged with infectious HSV after immunization with vaccinia virus recombinants expressing HSV gB or gD (Bernstein et al., 1988). Among the VZV glycoproteins, gpV appears to be unusual in its variable synthesis by VZV strains and the fact that the gpV gene product of some isolates is truncated as a result of deletions from a GC-rich repeat region (Kinchington et al., 1986, 1990). The limited host response generated by gpV in the guinea-pig model is consistent with the observation that some isolates of the Oka strain, used to produce the live attenuated varicella vaccine, make little or no gpV, whereas this investigational vaccine reliably induces protective immunity in healthy children and adults (Kinchington et al., 1990). Of note, the failure of vac-gpV to prevent VZV replication paralleled the lower virus-specific T lymphocyte response observed among animals given vac-gpV compared to those given vac-gpI or vac-gpIV. Similarly, protection against HSV challenge after immunization with vaccinia virus recombinants expressing HSV gD correlated with the induction of HSV-specific T lymphocyte proliferation (Wachsmann et al., 1987). Our experiments with vac-gpV also suggest differences in the potential of related VZV and HSV glycoproteins to induce antiviral immunity since the HSV homologue, gC, was protective in mice (Roberts et al., 1985). The HSV homologues of gpI and gpIV, which are gE and gI, respectively, have been expressed in vaccinia virus recombinants and evaluated in a murine HSV model (Blacklaws et al., 1990). In these experiments, immunization with gE increased the rate of virus clearance and reduced acute but not latent infection of ganglia; gI did not induce any protection. Further comparisons cannot be made at present because the HSV gD has no VZV homologue, and protection by VZV gpII, which is related to HSV gB, has not been investigated.

As noted in strain 2 guinea-pigs given immunoaffinity-purified gpI or IE-62 protein, the initial exposure to VZV gpI, gpIV, gpV or IE-62 expressed by vaccinia virus recombinants primed the host response of strain 2 guinea-pigs so that a significant boost in cell-mediated immunity was elicited by inoculation with guinea-pig cell-adapted VZV (Arvin et al., 1987). Enhanced T cell proliferation could be attributed to the failure of vac-gpV immunization to restrict the replication of the challenge virus. However, boosting also occurred in animals given vac-gpI, vac-gpIV and vac-IE-62. Past experiments in human subjects with natural immunity to VZV indicate that cell-mediated immunity is enhanced by close re-exposure to the virus (Arvin et al., 1983). This finding is relevant to vaccine development since it indicates that re-exposure to wild-type virus may also serve to maintain virus-specific immunity that was initially generated by immunization with a single VZV protein.

An important issue in herpesviral vaccine development is whether viral proteins expressed by infectious virus vectors, such as vaccinia virus, are more effective in eliciting sustained virus-specific immunity, especially cellular immunity, than purified protein preparations. Our experiments comparing vac-gpI and vac-IE-62 with the corresponding immunoaffinity-purified proteins indicate that immunity to these proteins produced by recombinant vaccinia virus, which involves de novo protein synthesis and antigen presentation occurring in infected host cells, was equivalent to the humoral and cell-mediated immunity elicited by relatively high concentrations of purified gpI or IE-62. Purified preparations of HSV and cytomegalovirus glycoproteins have also been shown to induce virus-specific antibodies and T lymphocyte proliferation (Mertz et al., 1984; Berman et al., 1985; Gönczöl et al., 1986; Meignier et al., 1987; Stanberry et al., 1987; Bernstein et al., 1988). Nevertheless, the production of purified proteins in sufficient quantity can be difficult and protein vaccines must incorporate adjuvants that are safe for human use. If the safety of vaccinia virus vectors is improved by inactivating viral genes such as ribonucleotide reductase or thymidine kinase, these animal experiments with VZV–vaccinia virus recombinants indicate that immunity to VZV proteins could be elicited by such a vaccine (Buller et al., 1985; Child et al., 1990).

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