Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 3896–3900, June 1987
Microbiology

Varicella–zoster virus as a live vector for the expression of foreign genes

(Epstein–Barr virus/recombinant vector/Oka strain)

ROBERT S. LOWE*, PAUL MALCOLM KELLER*, BERNICE J. KEECH*, ANDREW J. DAVISON†, YOUNG WHANG‡, ANDREW J. MORGAN‡§, ELLIOTT KIEFF‡, AND RONALD W. ELLIS*¶

*Department of Virus and Cell Biology, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486; †Medical Research Council Virology Unit, Institute of Virology, Glasgow, United Kingdom; and ‡Department of Medicine, University of Chicago, Chicago, IL 60637

Communicated by Edward M. Scolnick, February 9, 1987

ABSTRACT **The previous demonstration of the efficacy and tolerability of the Oka strain of varicella–zoster virus (VZV) in clinical trials involving vaccination of both normal and immunocompromised individuals has laid the foundation for its use in preventing chickenpox. In this context, VZV could be useful as a vector for vaccinating against other infectious agents as well. As an initial application, a live recombinant VZV expressing Epstein–Barr virus (EBV) membrane glycoproteins (gp350/220) was generated by inserting a gene fusion of the VZV gpI promoter and hydrophobic leader-encoding sequence with the gp350/220 coding sequence into the thymidine kinase (*TK*) gene of VZV(Oka). Insertion of the foreign DNA into the thymidine kinase gene was demonstrated by Southern blot analysis and the ability of the recombinant virus to replicate in the presence of bromodeoxyuridine. RNA splicing, glycosylation, and plasma membrane presentation of gp350/220 in cells infected with the recombinant virus were similar to those seen in EBV-infected cells. In addition, the expression of VZV-specific glycoproteins was unaltered by the concomitant expression of this large foreign glycoprotein. Thus, VZV can be used as a live viral vector for active immunization against EBV and other pathogens.**

Varicella–zoster virus (VZV) is an Alphaherpesvirus and the etiological agent of chickenpox and zoster (shingles) (1). Most people are infected with VZV by age 20, and, after a variable latent period, reactivation of the virus causes an estimated 5 million cases of zoster worldwide each year (2). VZV causes a relatively benign disease in healthy children. However, it can be morbid in adults and life-threatening to neonates (3, 4) as well as to immunocompromised patients with leukemia (5, 6), bone marrow transplants (7), and lymphoma (8, 9). An attenuated strain (Oka) of VZV has been developed for use as a live viral vaccine (10). It has been tested clinically in several thousand normal children as well as in hundreds of children with acute lymphocytic leukemia (henceforth referred to as immunocompromised, as a consequence of chemotherapy) and has proven in clinical trials to be both well tolerated and effective in preventing chickenpox (11, 12). Moreover, VZV(Oka) is the only live viral vaccine approved for use in such immunocompromised patients. For these reasons, we decided to test the feasibility of using VZV(Oka) as a live viral vector capable of expressing heterologous antigens and thereby protecting against other common human pathogens.

Epstein–Barr virus (EBV) is a lymphotropic Gammaherpesvirus that infects almost the entire population of the world. Primary infection with EBV usually results in mild disease but may lead to life-threatening complications in immunocompromised individuals (13, 14). In contrast, primary infection of adolescents can result in infectious mononucleosis (15). EBV is etiologically associated with Burkitt lymphoma and undifferentiated nasopharyngeal carcinoma; the latter affects up to 2% of the male population in southern China and other areas within Africa and Asia (16, 17). The possibility of protecting individuals from developing Burkitt lymphoma or nasopharyngeal carcinoma by preventing primary EBV infection through vaccination has been proposed (18). Moreover, the development of such a vaccine might result in prevention of infectious mononucleosis. However, EBV is intractable to its development as a live viral vaccine. EBV replicates poorly *in vitro* and efficiently transforms human B lymphocytes by mechanisms that are incompletely understood (19). Thus, EBV is a prototype agent for which a recombinant live viral vaccine would be appropriate; initial experiments have been carried out using vaccinia virus as a vector (20). The two major envelope glycoproteins of M_r 350,000 and 220,000 (gp350 and gp220) of EBV are derived from a single gene (21) by splicing out a portion of the protein coding sequence for gp350 to yield gp220 without a change in reading frame. Because of the abundance of EBV gp350/220 on the outer surface of virions and virally-infected cells (22, 23) and the role of these glycoproteins in inducing neutralizing antibodies (24, 25, 32, 54), gp350/220 are likely to be important in immunity to EBV infection. Therefore, we sought to create a VZV(Oka)–EBV gp350/220 recombinant virus that could be evaluated as a prototype VZV(Oka)-based recombinant live viral vaccine.

MATERIALS AND METHODS

Cells and Virus. The MRC-5 cell strain of human diploid fibroblasts (26) was maintained in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% fetal bovine serum (FBS), 100 units of penicillin per ml, and 100 μg of streptomycin per ml (growth medium). Attenuated VZV-(Oka) (10) was propagated by infection of MRC-5 cells with either cell-associated (infected MRC-5 cells) or cell-free virus.

Construction of a Recombinant VZV. The construction of an expression cassette [VZV P,L(gpI)-gp350], containing the promoter (P) and hydrophobic leader (L) sequence of VZV gpI adjacent to the gp350/220 coding sequence, is described elsewhere in detail (55). This plasmid potentially encodes a fusion protein containing the first 36 amino acids of VZV gpI and all but the first 20 amino acids of gp350/220. VZV

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: VZV, varicella–zoster virus; EBV, Epstein–Barr virus; TK, thymidine kinase; FBS, fetal bovine serum; mAb, monoclonal antibody; ORF, open reading frame.

§Present address: Department of Pathology, University of Bristol, United Kingdom.
¶To whom requests for reprints should be addressed.

P,L(gpI)-gp350 was cloned into the unique *Nsi* I site (27) of the VZV thymidine kinase (*TK*) gene (28) within the cloned *Sac* I H (29) fragment of VZV, creating pPK-4 (Fig. 1). Calcium phosphate precipitates (30) containing 1 μg of pPK-4 and 1 μg of VZV(Oka) genomic DNA (31) were added to 35-mm plates containing 3×10^5 MRC-5 cells. After 4 hr of incubation at 37°C, the cells were rinsed with growth medium, exposed to 15% glycerol for 3 min, again rinsed, and incubated for an additional 24 hr. The cells from each plate were trypsinized and passaged into two 60-mm plates. Five to ten days later, cells showing cytopathic effects were passaged to fresh MRC-5 cells and screened for recombinant virus by an indirect immune hemadsorption assay—i.e., erythrocyte rosetting (see below). Positive plaques were picked by cylinder cloning of infected cells, and virus was grown by serial passage in MRC-5 cells. Cell-free virus was isolated by sonicating infected cells for 30 sec, followed by freeze-thaw and plaque-purification in 96-well microtiter plates containing MRC-5 cells.

Serological Assays. Infected MRC-5 cells were incubated with growth medium containing 10 μg of murine monoclonal antibody (mAb) C1.4 (32) per ml to gp350/220 (Atlantic Antibodies, Westbrook, ME) for 1 hr at 37°C. The cells were washed three times with growth medium and incubated for 1 hr in growth medium containing human erythrocytes to which rabbit anti-mouse immunoglobulin had been coupled (33). The cells then were washed three times and screened visually for the formation of erythrocyte rosettes on VZV plaques. Double-label immunofluorescence was performed by incubating MRC-5 cells infected with recombinant VZV or parental VZV(Oka) for 1 hr in growth medium containing murine anti-gp350 mAb (mAb 2L10 and C1.4 were gifts from G. Pearson) and polyclonal human anti-VZV (EBV-negative) antibody. The cells then were washed, incubated for 1 hr in growth medium containing fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Tago, Burlingame, CA) and rhodamine-conjugated goat anti-human IgG (Cappel Laboratories, Cochranville, PA), washed again, and examined by fluorescence microscopy (Zeiss). For immunoblot, lysates from infected MRC-5 cells were prepared (34), and proteins were electrophoresed in 6% $NaDodSO_4$/polyacrylamide gels and electroblotted to nitrocellulose (35). The nitrocellulose filter was placed in buffer A (150 mM NaCl/50 mM Tris, pH 7.6/0.1% NaN_3/0.1% Tween 20) with 20% FBS for 24 hr at 4°C and then incubated with either human anti-EBV or human anti-VZV IgG in buffer A with 3% FBS for 12–16 hr at 4°C. The filters then were washed for 2 hr at 37°C in buffer A, placed in buffer A with 3% FBS and 0.25 μCi of ^{125}I-labeled protein A per ml (1 Ci = 37 GBq) for 2 hr at room temperature, washed, and exposed to film.

Immune-Electron Microscopy. mAb 2L10 was coupled to 12-nm colloidal gold (36). Infected MRC-5 cells were washed once and incubated with the colloidal gold-coupled 2L10 antibody (60 μg/ml) in growth medium at 37°C for 1 hr. Cells were washed, fixed, and processed for electron microscopy. For detection of VZV glycoproteins, infected cells were incubated first with anti-gpI mAb C1 (34), then with 5-nm colloidal gold-coupled goat anti-mouse IgG.

DNA Restriction Analysis of Recombinant VZV Virus. VZV genomic DNA digested with *Sac* I or *Bgl* II was electrophoresed on 0.8% agarose gels. Digested DNA fragments were transferred to nitrocellulose and probed with nick-translated [α-^{32}P]dCTP-labeled DNA from either the VZV *Sac* I H fragment or the EBV gp350/220 open reading frame (ORF).

RESULTS

Construction of an Expression Plasmid and Viral Recombinant. VZV expresses three major glycoproteins on the virion envelope—gpI, gpII, and gpIII; the genes for all three have been mapped and sequenced (37–39). Of these glycoproteins, gpI is apparently the most abundant (34) and is expressed at relatively high levels late during viral infection. Therefore, we chose to utilize the VZV gpI promoter to express non-VZV glycoproteins in a recombinant VZV. The salient features of the DNA sequence for this region include a CAAT–90 base pairs (bp)–TATAA–60 bp–ACCATGG–gpI ORF organization, suggesting that gpI mRNA is a nonspliced transcript regulated by a contiguous upstream promoter. To favor its recognition and processing as a VZV gene product, the EBV gp350/220 gene beginning at its 21st codon was fused in-frame to codon 35 of the putative gpI promoter-mRNA leader-gpI ORF fragment. Because cotransfected herpesvirus DNAs characteristically undergo homologous recombination with a high frequency, VZV gpI–EBV gp350/220 inserted into the VZV *TK* ORF (pPK-4) might be expected to recombine with cotransfected VZV genomic DNA through the homologous flanking *TK* DNA (Fig. 1). Indeed, cotransfection of MRC-5 cells resulted in 10% of the resulting foci being positive for the expression of gp350/220 as manifested by indirect immune hemadsorption. No background of positive foci was found when the pPK-4 DNA was omitted from the cotransfections or when an irrelevant mAb was used in the immune hemadsorption test. Three independent recombinant viral isolates (designated E/V-1, E/V-2, and E/V-3) were plaque-purified and further characterized. The recombinant virus developed plaques on MRC-5 cells at a rate similar to parental VZV(Oka). Parental and recombinant viruses also grew to comparable titer in MRC-5 cells. Thus, expression of gp350/220 did not interfere overtly with VZV replication.

Characterization of the Recombinant VZV Genome. In order to confirm that the gpI-gp350/220 gene fusion had recombined properly into the VZV(Oka) *TK* gene, analyses of recombinant viral isolate E/V-1 DNA were done. *Bgl* II digestion of VZV(Oka) DNA generates 20 fragments (29), with the fourth largest (*Bgl* II D fragment) containing the *TK* gene (27). Because there are no *Bgl* II sites within the 3.7-kilobase pair (kbp) gpI-gp350/220 gene fusion (Fig. 1), the *Bgl* II D fragment of the parental virus (seen as the *Bgl* II D/E doublet, Fig. 2*A*, arrow 2) was increased in size by 3.7 kbp (Fig. 2*A*, arrow 1). A VZV *TK* probe hybridized to the parental and recombinant fragments (Fig. 2*B*). Digestion of the VZV parental DNA with *Sac* I yielded the expected fragments including the *Sac* I H fragment, which contains the *TK* gene (Fig. 2*D*, arrow 4). Because there is a single *Sac* I site within the gpI-gp350/220 DNA (Fig. 1), cleavage of VZV recombinant DNA with *Sac* I yielded two other fragments (Fig. 2*D*, arrows 3 and 5). These two recombinant fragments hybridized to probes for VZV *Sac* I H and gp350/220 (Fig. 2 *E* and *F*). The sizes of the recombinant VZV *Sac* I fragments are identical to those of the cotransfected recombinant plasmid pPK-4, indicating that there was no substantial insertion or deletion at the sites of recombination and that the gpI-gp350/220 insert had retained the same orientation in the recombinant VZV genome as it had in pPK-4.

The parental VZV did not grow in a TK^- cell line (A673) in the presence of 100 μg of bromodeoxyuridine per ml, since the viral TK protein phosphorylates the bromodeoxyuridine, which then becomes incorporated into viral DNA. In contrast, the recombinant VZV isolates grew in the TK^- cells in the presence of bromodeoxyuridine, indicating that the insertional inactivation of *TK* gene by homologous recombination with the gpI-gp350 gene fusion had occurred.

Immunological Characterization of the Recombinant Virus by Microscopy. Double immunofluorescent labeling demonstrated expression of both VZV glycoproteins and gp350/220 on the surface of infected cells (Fig. 3). However, electron-microscopic examination of immunogold-labeled cells infected by recombinant virus revealed a qualitative difference in

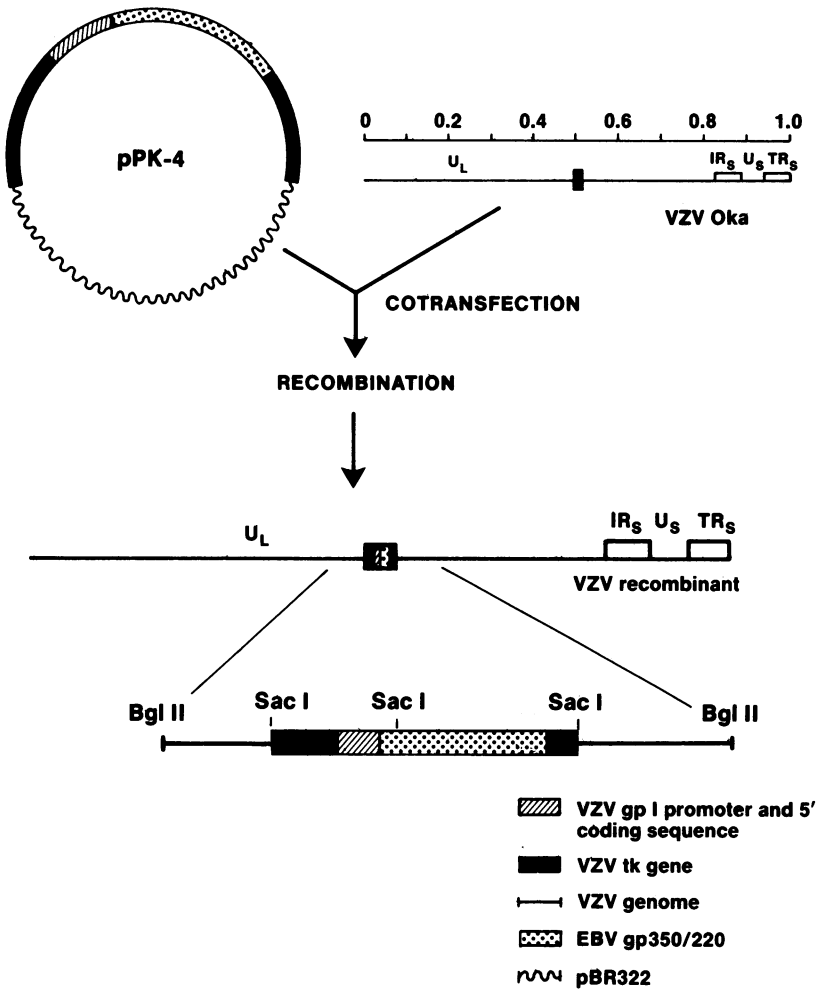

FIG. 1. Construction of live recombinant VZV expressing gp350/220. Major structural features: long unique region (U_L), short inverted repeat (IR_s), short unique region (U_s), and short terminal repeat region (TR_s) of the VZV genome are indicated and aligned with a scale of fractional genome length. The organization of pPK-4 is described in *Materials and Methods*.

the pattern of distribution of VZV gpI and EBV gp350/220 (Fig. 4). Gold-labeled anti-gpI mAb primarily reacted with mature virions in the extracellular space; less antibody reacted with the cellular plasma membrane. In contrast, anti-gp350 mAb primarily localized to the plasma membrane; less than 10% of virions were labeled. Thus, even though the recombinant gp350 is a fusion protein, the amino terminus of which is predicted to be 35 amino acids of gpI, the gp350 coding domain is the important determinant of the fate of the molecule in the surface of the infected cell. The small degree of anti-gp350 labeling of recombinant virus was significant, because the antibody did not bind to virus or cells infected with parental VZV. The density of VZV gpI as well as the morphology and size of the virions appear similar in both the parental and recombinant virus.

Recombinant VZV Glycoprotein Expression. The expression of VZV glycoproteins was not affected by insertion of the gpI-gp350/220 gene fusion into the VZV *TK* gene. Recombinant VZV isolates expressed VZV glycoproteins at the same level as the parental VZV (Fig. 5, lanes 6–8). The recombinant virus also expressed high levels of gp350/220, whereas no gp350/220 cross-reactive material was detected in parental VZV-infected cells (lanes 2–4). The gp350/220 comigrated with antigens expressed in Vero cells transformed with an expression vector (lane 1).

DISCUSSION

We have demonstrated the use of VZV as a live viral vector for the efficient expression of EBV gp350/220. One major advantage of using a live viral vector to immunize against heterologous pathogens is the ability of the virus to express, modify, and present antigens as they are expressed during the course of natural infection. Post-translational modifications, such as proper protein folding, glycosylation, and membrane insertion and presentation, may play critical roles in the ability of the processed protein to elicit a long-lasting and neutralizing immune response. For instance, glycosylation has been shown to influence the recognition by the immune system of influenza virus hemagglutinin and murine leukemia virus gp70 glycoproteins (40). The presentation of a protein also can greatly influence its immunogenicity (41–43). While neither processing nor presentation may be necessary for the production of neutralizing antibodies in every case, the epitopes needed for protective immune responses against a particular pathogen may be in question. The use of a live viral vector would address some of these concerns.

A second major advantage of a live recombinant viral vaccine is the ability of the virus to replicate in the host. This amplifies the expression of the heterologous proteins, often increasing both humoral and cellular immune responses to that antigen (44). Moreover, the expression of a viral antigen in association with class I histocompatibility antigens at the surface of infected cells enables recognition of the foreign antigen by cytotoxic T cells (45). The use of a very active promoter sequence, such as gpI, adjacent to the coding region of the heterologous gene also may increase the amount of protein expressed.

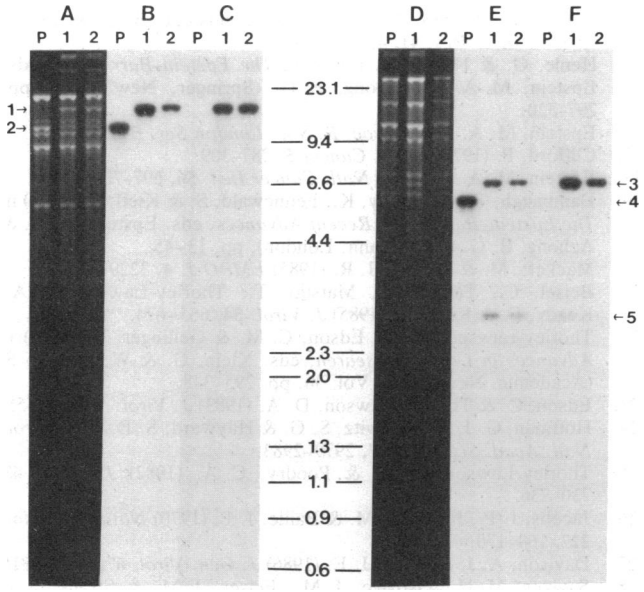

FIG. 2. Analysis of parental and recombinant viral DNA. DNAs isolated from VZV(Oka), recombinant viral isolate E/V-1, or recombinant viral isolate E/V-2 (labeled P, 1, and 2, respectively) were digested with *Bgl* II (*A–C*) or *Sac* I (*D–F*) and electrophoresed in agarose gels. After visualization with UV light (*A* and *D*), the DNA fragments were transferred to nitrocellulose and probed with radiolabeled DNA from either the VZV *Sac* I H fragment (*B* and *E*) or the entire gp350/220 coding region (*C* and *F*). Molecular mass markers are in kbp.

VZV has the smallest genome among human Alphaherpesviruses. Because its virion is similar in size to those of cytomegalovirus and herpes simplex virus, it is likely that VZV could carry significant amounts of heterologous genetic information without selection against the larger DNA. Moreover, like other herpesviruses, VZV likely has significant parts of its genome that are dispensable without interference with the ability of the virus to replicate. Thus, significant parts of the genome can be deleted, creating additional space for heterologous genes.

The use of VZV as a viral vector provides many distinct advantages over other live viral vectors for the expression of foreign genes. Viruses from live viral vaccines have been

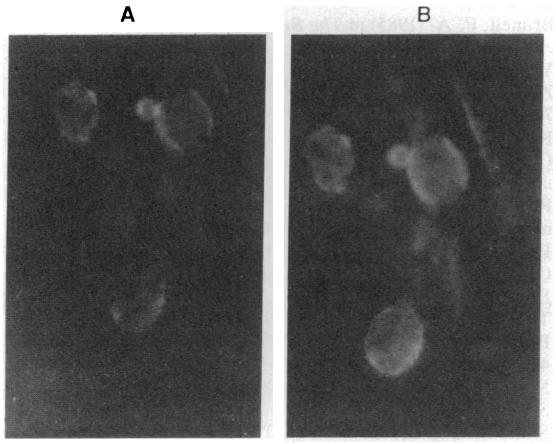

FIG. 3. Double immunofluorescent labeling of MRC-5 cells infected with recombinant viral isolate E/V-1. Infected cells were incubated with murine anti-gp350 mAb and human anti-VZV polyclonal convalescent serum. They subsequently were incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG and rhodamine-conjugated goat anti-human IgG. Excitation with a fluorescence-specific light source of narrow bandwidth allows visualization of gp350-specific fluorescence (*A*) or VZV-specific fluorescence (*B*).

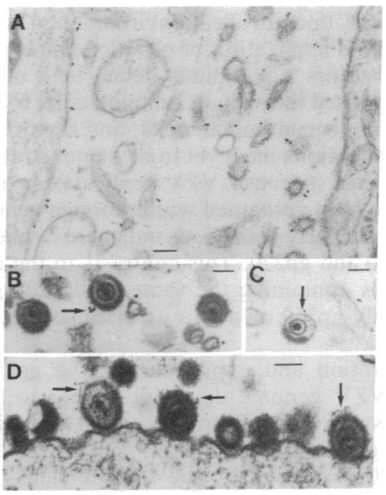

FIG. 4. Immune-electron microscopy of recombinant VZV. (*A–C*) Recombinant viral isolate E/V-1-infected cells treated with anti-gp350 mAb coupled to colloidal gold. (*A*) Gold particles label the infected cellular plasma membranes, indicating the presence of gp350/220. (*B* and *C*) Arrows indicate gold-labeled mature virions. (*D*) gpI on the virion envelope is detected by mAb to gpI and gold-coupled goat anti-mouse IgG. Arrows, gold particles labeling the virion envelope. Size bars, 100 nm.

transmitted inadvertently to immunocompromised individuals through contact with recently vaccinated individuals, and, in the case of vaccinia and polio, such transmission has had severe consequences (44, 46, 47). This should not be a concern with VZV(Oka), since its efficacious use has been demonstrated in clinical trials in immunocompromised individuals (11). VZV(Oka) is the only live viral vaccine currently licensed for use in immunocompromised individuals, making it an extremely attractive virus for use as a vector to vaccinate the general population against other serious diseases. Other viruses proposed for use as vectors contain genetic elements with the capacity of cellular transformation (48) or mitogenesis through binding to cellular receptors for growth factors (49, 50). Unlike other viral vectors (51–53) that have a wide host range and potentially could be transmitted horizontally between humans and other species, the host range of VZV is limited essentially to humans, thus minimiz-

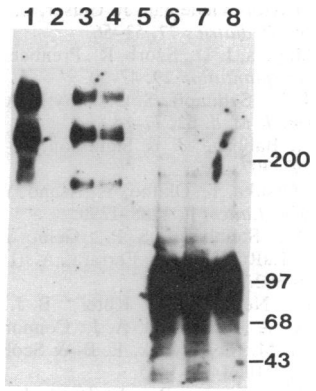

FIG. 5. Immunoblot of parental and recombinant viral proteins. Lysates from MRC-5 cells infected with parental VZV(Oka) (lanes 2 and 6) or recombinant viral isolate E/V-1 (lanes 3, 4, 7, and 8) or immunoaffinity-purified gp350/220 from a stably transfected Vero cell line (lanes 1 and 5; ref. 55) were electrophoresed on 6% polyacrylamide gels, electro-blotted to nitrocellulose, and incubated with anti-EBV (lanes 1–4) or anti-VZV (lanes 5–8) polyclonal sera followed by incubation with ^{125}I-labeled Protein A. Molecular mass markers are in kDa.

ing the risk of uncontrolled environmental spread. It is possible that the application of genetic engineering to these other viral vectors could limit deleterious effects in immunocompromised individuals, eliminate the risk of adverse interactions in normal human cells, and restrict host range, but these applications have yet to be demonstrated. It also is noteworthy that, because VZV replicates in the nucleus, mRNA splice sites contained within foreign gene transcripts appear to be properly spliced, as indicated by the production of both gp350 and gp220. This contrasts to the recombinant vaccinia virus containing the same gp350/220 gene, which expresses only gp350 owing to its cytoplasmic site of replication (20).

The integration and expression of the gp350/220 gene within the VZV genome does not appear to alter overtly either expression or incorporation within the virion envelope of the major glycoproteins of VZV. At the same time, the size of gp350/220 expressed by the recombinant virus and detected within the plasma membranes of infected fibroblasts provides preliminary evidence for correct post-translational modifications and presentation of these heterologous glycoproteins. The growth characteristics of the recombinant virus and its ability to elicit a protective immune response in an animal model system deserve further investigation.

The ultimate use of a live viral vaccine will be determined by the perceived risk involved in vaccinating against heterologous pathogens compared to the risk involved in contracting the natural disease. The efficacious and well-tolerated use of VZV(Oka) in vaccinating both normal and immunocompromised individuals lays the foundation for its use as a live recombinant viral vector for protecting against both chickenpox and other human diseases.

We gratefully acknowledge M. Armstrong, E. Emini, E. Scolnick, and M. Silberklang for helpful discussions, E. Emini and G. Pearson for reagents, and G. Albanesius for careful preparation of the manuscript.

1. Takahashi, M. (1983) *Adv. Virus Res.* **28,** 285–356.
2. Plotkin, S. A., Arbeter, A. A. & Starr, S. E. (1985) *Postgrad. Med. J.* **61,** 155–162.
3. Hanngren, K., Grandien, M. & Granstrom, G. (1985) *Scand. J. Infect. Dis.* **17,** 343–347.
4. Paryani, S. G. & Arvin, A. M. (1986) *N. Engl. J. Med.* **314,** 1542–1546.
5. Feldman, S., Hughes, W. T. & Kim, H. Y. (1973) *Am. J. Dis. Child.* **126,** 178–184.
6. Brunell, P. A., Taylor-Wiedeman, J., Geiser, C. F., Frierson, L. & Lydick, E. (1986) *Pediatrics* **77,** 53–56.
7. Atkinson, K., Meyers, J. D., Szorb, R., Prentice, R. L. & Thomas, E. D. (1980) *Transplantation* **29,** 47–50.
8. Ruckdeschel, J. C., Schimpff, S. C., Smyth, A. C. & Mardiney, M. R. (1977) *Am. J. Med.* **62,** 77–85.
9. Souhami, R. L., Babbage, J. & Sigfusson, A. (1983) *Clin. Exp. Immunol.* **53,** 297–307.
10. Takahashi, M., Otsuka, T., Okuno, Y., Asano, Y., Takehiko, Y. & Isomura, S. (1974) *Lancet* **ii,** 1288–1290.
11. Gershon, A. A., Steinberg, S. P., Gelb, L., Galasso, G., Borowsky, W., LaRussa, P. & Ferrara, A. (1984) *J. Am. Med. Assoc.* **252,** 355–362.
12. Weibel, R. A., Neff, B. J., Kuter, B. J., Guess, H. A., Rothenberger, C. A., Fitzgerald, A. J., Connor, K. A., McLean, A. A., Hilleman, M. R., Buynak, E. B. & Scolnick, E. M. (1984) *N. Engl. J. Med.* **310,** 1409–1415.
13. Masucci, M. G., Szigeti, R., Ernberg, I., Bjorkholm, M., Mellstedt, H., Henle, G., Henle, W., Pearson, G., Masucci, G., Svedmyr, E., Johansson, B. & Klein, G. (1981) *Cancer Res.* **41,** 4292–4301.
14. Purtilo, D. T., Sakamoto, K., Saemundsen, A. K., Sullivan, J. L., Synnerholm, A., Anvret, M., Prichard, J., Sloper, C., Sieff, C., Pincott, J., Pachman, L., Rich, K., Cruzi, F., Cornet, J. A., Collins, R., Barnes, N., Knight, J., Sandstedt, B. & Klein, G. (1981) *Cancer Res.* **41,** 4226–4236.
15. Henle, G. & Henle, W. (1979) in *The Epstein–Barr Virus*, eds. Epstein, M. A. & Achong, B. G. (Springer, New York), pp. 297–320.
16. Epstein, M. A. (1984) *Proc. R. Soc. London Ser. B* **221,** 1–20.
17. Clifford, P. (1970) *Int. J. Cancer* **5,** 287–309.
18. Epstein, M. A. (1976) *J. Natl. Cancer Inst.* **56,** 697–700.
19. Dambaugh, T., Hennessy, K., Fennewald, S. & Kieff, E. (1986) in *The Epstein–Barr Virus: Recent Advances*, eds. Epstein, M. A. & Achong, B. G. (Heinemann, London), pp. 13–45.
20. Mackett, M. & Arrand, J. R. (1985) *EMBO J.* **4,** 3229–3234.
21. Beisel, C., Tanner, J., Matsuo, T., Thorley-Lawson, D. A., Kezdy, F. & Kieff, E. (1985) *J. Virol.* **54,** 665–674.
22. Thorley-Lawson, D. A., Edson, C. M. & Geilinger, K. (1982) in *Advances in Cancer Research*, eds. Klein, G. & Weinhouse, S. (Academic, New York), Vol. 36, pp. 295–348.
23. Edson, C. & Thorley-Lawson, D. A. (1983) *J. Virol.* **46,** 547–555.
24. Hoffman, G. J., Lazarowitz, S. G. & Hayward, S. D. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2979–2983.
25. Thorley-Lawson, D. A. & Poodry, C. A. (1982) *J. Virol.* **43,** 730–736.
26. Jacobs, J. P., Jones, C. M. & Baille, J. P. (1970) *Nature (London)* **227,** 168–170.
27. Davison, A. J. & Scott, J. E. (1986) *J. Gen. Virol.* **67,** 1759–1816.
28. Sawyer, M. H., Ostrove, J. M., Felser, J. M. & Straus, S. E. (1986) *Virology* **149,** 1–9.
29. Davison, A. J. & Scott, J. E. (1983) *J. Gen. Virol.* **64,** 1811–1814.
30. Lowy, D. R., Rands, E. & Scolnick, E. M. (1978) *J. Virol.* **26,** 291–298.
31. Straus, S. E., Aulakn, H. S., Ruyechan, T., Hay, J., Casey, T. A., Vande Woude, G. F., Owens, J. & Smith, H. A. (1981) *J. Virol.* **40,** 516–525.
32. Thorley-Lawson, D. A. & Geilinger, K. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5307–5311.
33. Littman, D. R., Thomas, Y., Maddon, P. J., Chess, L. & Axel, R. (1985) *Cell* **40,** 237–246.
34. Keller, P. M., Neff, B. J. & Ellis, R. W. (1984) *J. Virol.* **52,** 293–297.
35. Burnette, W. N. (1981) *Anal. Biochem.* **112,** 195–203.
36. Slot, J. W. & Geuze, H. J. (1985) *Eur. J. Cell Biol.* **38,** 87–93.
37. Ellis, R. W., Keller, P. M., Lowe, R. S. & Zivin, R. A. (1985) *J. Virol.* **53,** 81–88.
38. Keller, P. M., Davison, A. J., Lowe, R. S., Bennett, C. D. & Ellis, R. W. (1986) *Virology* **152,** 181–191.
39. Keller, P. M., Davison, A. J., Lowe, R. S., Riemen, M. W. & Ellis, R. W. (1987) *Virology* **157,** 526–533.
40. Alexander, S. & Elder, J. H. (1984) *Science* **226,** 1328–1330.
41. Almeida, J. D., Edwards, D. C., Brand, C. M. & Heath, T. D. (1975) *Lancet* **ii,** 899–901.
42. Manesis, E. K., Cameron, C. H. & Gregoriadis, G. (1979) *FEBS Lett.* **102,** 107–111.
43. Sakai, F., Gerlier, D. & Dore, J. F. (1980) *Br. J. Cancer* **41,** 227–235.
44. Brunell, P. A. (1985) in *The Basis for Serodiagnosis and Vaccines*, eds. Van Regenmortel, M. H. V. & Neurath, A. R. (Elsevier, New York), pp. 171–188.
45. Zinkernagel, R. M. & Doherty, P. C. (1974) *Nature (London)* **248,** 701–702.
46. Kempe, H. (1960) *Pediatrics* **26,** 176–189.
47. Centers for Disease Control (1982) *Poliomyelitis Surveillance Summary 1980–1981* (Centers for Disease Control, Atlanta).
48. Rawls, W. E. (1985) in *The Herpesviruses*, ed. Roizman, B. (Plenum, New York), Vol. 3, pp. 241–255.
49. Brown, J. P., Tusardzik, D. R., Marquardt, H. & Todaro, G. J. (1985) *Nature (London)* **313,** 491–492.
50. Eppstein, D. A., Marsh, Y. V., Schreiber, A. B., Newman, S. R., Todaro, G. J. & Nestor, J. J. (1985) *Nature (London)* **318,** 663–665.
51. Panicali, D. & Paoletti, E. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4927–4931.
52. Mackett, M., Smith, G. L. & Moss, B. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7415–7419.
53. Shih, M. F., Arsenakis, M., Tiollais, P. & Roizman, B. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5867–5870.
54. Strnad, B. C., Schuster, T., Klein, R. & Hopkins, R. F. (1982) *J. Virol.* **41,** 258–264.
55. Whang, Y., Silberklang, M., Morgan, A., Munshi, S., Lenny, A. B., Ellis, R. W. & Kieff, E. (1987) *J. Virol.*, in press.