Immunization with Recombinant Varicella-Zoster Virus Expressing Herpes Simplex Virus Type 2 Glycoprotein D Reduces the Severity of Genital Herpes in Guinea Pigs

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Varicella-zoster virus (VZV) is an attractive candidate for a live-virus vector for the delivery of foreign antigens. The Oka vaccine strain of VZV is safe and effective in humans, and recombinant Oka VZV (ROka) can be generated by transfecting cells with a set of overlapping cosmid DNAs. By this method, the herpes simplex virus type 2 (HSV-2) glycoprotein D (gD2) gene was inserted into an intergenic site in the unique short region of the Oka VZV genome. Expression of gD2 in cells infected with the recombinant Oka strain VZV (ROka-gD2) was confirmed by antibody staining of fixed cells and by immunoblot analysis. Immune electron microscopy demonstrated the presence of gD2 in the envelope of ROka-gD2 virions. The ability of ROka-gD2 to protect guinea pigs against HSV-2 challenge was assessed by inoculating animals with three doses of uninfected human fibroblasts, fibroblasts infected with ROka VZV, or fibroblasts infected with ROka-gD2. Neutralizing antibodies specific for HSV-2 developed in animals immunized with ROka-gD2. Forty days after the third inoculation, animals were challenged intravaginally with HSV-2. Inoculation of guinea pigs with ROka-gD2 significantly reduced the severity of primary HSV-2 infection ($P < 0.001$). These experiments demonstrate that the Oka strain of VZV can be used as a live virus vector to protect animals from disease with a heterologous virus.

Live recombinant viruses expressing foreign antigens have potential advantages over subunit vaccines. The foreign antigens may be posttranslationally modified, as in natural infection, thus preserving their native tertiary structures, which may be important for eliciting protective humoral immunity. Expression of foreign antigens in the context of the major histocompatibility complex antigens may result in enhanced induction of cytotoxic T lymphocytes. Finally, infection of mucosal cells following inoculation with a live virus vaccine may elicit mucosal as well as systemic immune responses.

Varicella-zoster virus (VZV), the herpesvirus which causes chicken pox after initial infection and shingles upon reactivation from latency, is especially attractive as a live-virus vector. The live, attenuated Oka vaccine strain of VZV has been administered to more than a million people in Japan and Korea and has proven safe and effective in preventing chicken pox in both immunocompetent and immunocompromised persons (for a review, see reference 30). In addition, VZV Oka has recently been recommended for universal childhood immunization in the United States. VZV has several genes which can be interrupted without interfering with viral replication in cultured cells (6–9, 12, 13, 18, 23), thus offering potential sites for the insertion of foreign DNA into the genome. These genes may also be deleted to provide additional space for exogenous DNA. Furthermore, while VZV has the smallest genome of the human herpesviruses, its capsid is similar in size to that of herpes simplex virus (HSV) which has a larger genome; therefore, VZV may be able to accommodate a larger recombinant genome within its capsid.

To test whether VZV expressing a foreign antigen could be used as a live-virus vaccine to protect animals against challenge with a heterologous pathogen, the glycoprotein D (gD) gene of HSV type 2 (HSV-2) was inserted into the Oka VZV genome, and the recombinant VZV was used to immunize guinea pigs. Purified forms of gD have been the focus of intense study for use as immunogens in HSV-2 vaccines (for a review, see reference 24). Immunization of animals or humans with gD induces antibodies which neutralize HSV infectivity (5, 20, 29). gD is also a target of antibody-dependent cellular cytotoxicity and CD4- and CD8-specific cytotoxic T cells (15, 16). Animals immunized with purified forms of HSV-2 gD (gD2) (2, 25) or with vaccinia virus expressing gD2 (31) can be protected against challenge with HSV-2. In addition, gD2 immunization of people or guinea pigs with genital herpes reduces the frequency of recurrences (25, 28).

In this study, we showed that gD2 expressed by recombinant VZV Oka was present in the envelope of the recombinant virus and on the surfaces of virus-infected cells. Furthermore, we demonstrated that immunization of guinea pigs with recombinant Oka VZV expressing gD2 reduced the severity of primary genital herpes after challenge with HSV-2.

Generation of VZV recombinants containing the HSV-2 gD gene. Infectious recombinant VZV can be produced by cotransfection of four overlapping cosmids which span the entire viral genome (6) (Fig. 1). The HSV-2 gD coding sequences (19, 32) were incorporated into the Mst IIA cosmid under the control of either the endogenous gD2 promoter (cosmid Mst IIA-gD2/D) or under the control of the VZV gE promoter (cosmid Mst IIA-gD2/E [Fig. 1]). The HSV-2 gD gene under the control of its own promoter was generated by PCR amplification of HSV-2 (strain 333) DNA. The resulting DNA fragment (gD2/D) begins 200 bp upstream of the gD2 start codon and ends immediately following the gD2 poly(A) signal se-

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FIG. 1. Construction of recombinant VZV containing HSV-2 gD. (A) The prototype VZV genome (Dumas strain) is 124,884 bp in length (A) and consists of terminal repeats (TR), internal repeats (IR), and unique long (U_L) and unique short (U_S) DNA domains. (B and C) The four overlapping cosmids used to generate infectious virus. The cosmids are named according to the restriction fragments from which they were derived. The nucleotide coordinates correspond to the sequence of the prototype strain of VZV (10). (D) HSV-2 gD, under the control of either its endogenous promoter or the VZV gE promoter, was inserted into an intergenic *AvrII* site in the MstII A cosmid.

quence and has *AscI* restriction sites near both ends. The gD2/D fragment was ligated into the *AscI* site of plasmid pNEB193 (New England Biolabs, Beverly, Mass.), yielding p193-gD2/D. To express gD2 under the control of the VZV gE promoter, the gD2 coding sequences beginning immediately upstream of the HSV-2 (strain 333) gD start codon and ending immediately following the gD2 poly(A) signal sequence were amplified by PCR. The resulting DNA fragment (gD2/E) contains *PacI* and *AscI* restriction endonuclease sites at its 5' and 3' ends, respectively, and was ligated into pNEB193 at the *PacI* and *AscI* sites yielding plasmid p193-gD2. The VZV gE promoter was amplified by PCR from VZV Oka DNA. The resulting VZV DNA fragment extends from 803 to 9 bp upstream of the gE start codon and contains *AscI* and *PacI* restriction endonuclease sites near its 5' and 3' ends, respectively. The VZV gE promoter was inserted between the *PacI* and *AscI* sites of pNEB193, yielding plasmid p193-gE. Plasmid p193-gD2 was cut with *EcoRI*, blunt ends were generated with the Klenow fragment of DNA polymerase I, and the linearized plasmid was cut with *PacI*. The resulting 1.3-kb fragment containing pD2 was ligated into p193-gE, which had previously been cut with *HindIII*, blunted with Klenow, and cut with *PacI*, to yield plasmid p193-gD2/E. The gD2 genes including their promoters were excised from plasmids p193-gD2/D and p193-gD2/E with *AscI* and ligated into the *AvrII* site (nucleotide 112,853) of cosmid VZV MstII A, yielding cosmids MstII A-gD2/D and MstII A-gD2/E (Fig. 1). This *AvrII* site is located between VZV open reading frames (ORFs) 65 and 66, both of which are homologous to nonessential HSV-1 genes. These ORFs are transcribed in opposite directions, and the *AvrII* cloning site is 213 and 184 bp upstream from ORFs 65 and 66, respectively.

Transfection of MeWo cells (6) with cosmid MstII A-gD2/E or MstII A-gD2/D in addition to the other three parental cosmids resulted in plaques 9 days after transfection. Transfection of the four parental cosmids also resulted in plaques 9

FIG. 2. Southern blot analysis of recombinant VZV DNA. Virion DNAs from parental recombinant VZV (ROka), VZV containing gD2 driven by the HSV-2 gD promoter (ROka-gD2/D), and VZV containing gD2 driven by the VZV gE promoter (ROka-gD2/E) were isolated from nucleocapsid preparations, digested with *XhoI*, and probed with all four radiolabeled cosmid DNAs (A) or with DNA sequences specific for HSV-2 gD (B). The 13.8-kb *XhoI* restriction fragment containing the HSV-2 gD gene is present in ROka-gD2/D and ROka-gD2/E, but not in ROka (open arrow). The other *XhoI* restriction fragments of ROka-gD2/D and ROka-gD2/E are identical in size to those of ROka, demonstrating the absence of gross alterations in the VZV genome during recombination. Numbers refer to the sizes of DNAs in kilobase pairs.

days after transfection. Plaques produced in MeWo cells by the ROka-gD2/E or ROka-gD2/D recombinants were equivalent in size and morphology to those produced by parental recombinant VZV (ROka). Media overlying the cells infected with ROka or ROka-gD2 recombinants contained little or no cell-free virus. Thus, expression of HSV-2 gD by VZV did not

FIG. 3. Immunoblot analysis of cells infected with recombinant VZV expressing HSV-2 gD. Lysates of uninfected cells and cells infected with parental recombinant VZV (ROka), ROka containing the HSV-2 gD gene driven by its own promoter (ROka-gD2/D) or by the VZV gE promoter (ROka-gD2/E), or cells infected with HSV-2 were analyzed on immunoblots with antibody specific for HSV gD (A) or VZV gE (B). gD was expressed by both VZV recombinants containing the HSV-2 gD gene; however, HSV-2 gD was expressed at higher levels when driven by the endogenous HSV-2 promoter rather than the VZV gE promoter. VZV gE was expressed in similar amounts in cells infected with each of the VZV recombinants, indicating comparable levels of infection. Numbers refer to sizes of proteins in kilodaltons.

FIG. 4. Immune electron microscopy of VZV expressing HSV-2 gD. Cells infected with ROka-gD2/E (top) or ROka (bottom) were incubated with rabbit anti-HSV gD antibody (1:500 dilution) and then with colloidal gold-labeled anti-rabbit immunoglobulin G (1:30 dilution). The cells were fixed, sectioned, and examined by transmission electron microscopy. ROka-gD2/E virions and cells infected with ROka-gD2/E, but not ROka virions or their infected cells, show gold particles that are indicative of HSV-2 gD on their surfaces.

markedly affect the growth rate of the virus, nor did it enhance the ability of cells to produce infectious cell-free virus in vitro (14).

Southern blot analysis (Fig. 2) of viral DNA from infected cells verified the presence of gD2 sequences in the genomes of the two ROka-gD2 recombinants but not in that of the parental recombinant virus.

gD2 is expressed in cells infected by ROka-gD2. Cultured cells infected with HSV-2 express gD as a family of proteins ranging in size from 51 to 65 kDa (4). Expression of gD2 in MeWo cells infected with the ROka-gD2 recombinants was

analyzed by incubating blots containing equivalent amounts of total protein from infected-cell lysates with antibody specific for gD (Fig. 3A). Cells infected with ROka-gD2/D or ROka-gD2/E contained a family of gD2 proteins that were similar in size to those expressed during infection with HSV-2. Utilization of the VZV gE promoter to express gD2 as well as endogenous gE in ROka-gD/E raised the theoretical concern that cells infected with this recombinant might express smaller amounts of VZV gE than parental recombinant VZV. However, immunoblots of infected cell lysates incubated with antibody against VZV gE (Chemicon International, Inc., Te-

TABLE 1. Development of HSV-2-specific antibody titers following immunization with recombinant VZV expressing HSV gD2

Inoculum	HSV-2 neutralization titer ^b			
	Serum ^c no. 1	Serum no. 2	Serum no. 3	Serum no. 3 + comple- ment
Uninfected HFF cells	0	0	1:3	1:8
ROka-infected HFF cells	0	0	1:1	1:4
ROka-gD2/E-infected HFF cells	0	1:7	1:28	1:160

^a The animals were inoculated intramuscularly three times at 1-month intervals with infected cells. The animals received equivalent doses of uninfected HFF cells. NA, not applicable.

^b Values were calculated as 90% reduction in plaque numbers.

^c Serum was collected 3 weeks after each immunization.

mecula, Calif.) showed similar levels of gE expression during infection with ROka, ROka-gD2/E, and ROka-gD2/D (Fig. 3B). Indirect immunofluorescence showed gD2 in the cytoplasm and plasma membranes of cells infected with both ROka-gD2 recombinants (14).

gD2 is present in the envelope of ROka-gD2 virions. gD, which is located in the envelope of HSV-2 virions, plays a role in viral attachment and is essential for HSV-2 penetration of cells (11, 17). By immune electron microscopy, gD2 was demonstrated in the envelope of ROka-gD2/E virions (Fig. 4, top) and on the plasma membranes of cells infected with ROka-gD2/E. No gD2 was present in the envelope of the parental ROka virions or on the membranes of cells infected with ROka (Fig. 4, bottom). Maturation, budding and morphology of ROka-gD2/E virions were indistinguishable from ROka virions, suggesting that expression of gD2 on the surface of VZV virions results in no obvious changes in virion assembly or structure.

Immunization of guinea pigs with ROka-gD2 induces neutralizing antibodies specific for HSV-2. To determine whether gD2 was immunogenic when expressed by recombinant VZV, 3 groups of 12 guinea pigs each were inoculated intramuscularly 3 times at 1-month intervals with uninfected human fibroblasts (HFF cells), HFF cells containing 10^4 50% tissue culture infective dose (TCID₅₀) ROka VZV, or HFF cells containing 3.2×10^4 TCID₅₀ ROka-gD2/E. Although the titers of the ROka and ROka-gD2/E inocula were different, previous experiments with guinea pigs have shown that differences of this magnitude do not affect the immune responses to VZV (1, 21). Neutralizing antibody titers (defined as the concentration of guinea pig serum that reduced the number of plaques by 90% compared with preimmune guinea pig serum) were determined in the absence of complement for all guinea pigs (3) by using serum collected 3 weeks following each immunization

and in the presence of 10% (vol/vol) guinea pig complement for serum obtained after the third immunization (Table 1). After three immunizations with ROka-gD2/E, animals developed HSV-2-specific neutralizing titers (range, 1:16 to 1:64; mean, 1:28). The ability of immune serum to neutralize HSV-2 was enhanced (mean titer, 1:160) by the addition of complement.

Immunization of guinea pigs with ROka-gD2 reduces the severity of HSV-2 infection. Six weeks following the third immunization, the animals were challenged by intravaginal inoculation of 5.0×10^5 PFU of HSV-2 strain MS. Vaginal swabs were collected 1 day post-HSV-2 challenge, and the titers of virus that were assayed on rabbit kidney cells were similar for the three groups (Table 2). The animals were examined daily by an observer blinded to the animals' immunization group for 14 days following HSV-2 inoculation to assess the course of primary genital skin disease and from days 15 to 49 for evidence of recurrent disease. Primary genital disease was scored each day on a scale from 0 (no disease) to 4 (severe disease with large ulcers and maceration). Scores were averaged within each group (mean lesion score) and plotted against time following challenge, and the overall severity score was determined from the area under the curve (27).

All animals immunized with ROka-gD2/E developed skin lesions after challenge with HSV-2; however, the severity of disease was significantly reduced ($P < 0.001$) in animals immunized with ROka-gD2/E compared with that in animals receiving uninfected or ROka-infected cells. In addition, the incidence of hind limb paralysis was significantly less ($P < 0.005$) in animals immunized with ROka-gD2/E. Three animals immunized with uninfected cells, seven animals immunized with ROka-infected cells, and one animal immunized with ROka-gD2/E-infected cells died from primary HSV-2 disease. The limited number of control animals which could be evaluated following the resolution of primary HSV-2 infection precluded comparison of the incidence or severity of recurrent genital disease among the immunization groups; however, all ROka-gD2/E-immunized animals that could be evaluated developed recurrent genital disease.

We have shown that insertion of the HSV-2 gD gene into the VZV Oka genome results in the expression of gD2 in the virion envelopes and the plasma membranes of infected cells. Guinea pigs immunized with ROka-gD2-infected cells produced neutralizing antibodies specific for HSV-2. Although they were not protected from HSV-2 infection, animals immunized with ROka-gD2 experienced significantly less-severe primary disease and had a lower incidence of hind limb paralysis and mortality.

The level of protection from HSV-2 challenge observed in guinea pigs with live ROka-gD2 was less than that previously observed following immunization with subunit preparations of

TABLE 2. Clinical disease in vaccinated animals after intravaginal challenge with HSV-2

Inoculum	n	Incidence ^a	Severity ^b	Vaginal HSV-2 titer ^c	Hind limb paralysis	Mortality	Recurrent disease ^d
Uninfected HFF cells	12	8/10	10.81 $\pm$ 1.07	8.04 $\pm$ 1.00	6/11	3/11	3/4
ROka-infected HFF cells	12	10/11	10.95 $\pm$ 1.32	8.69 $\pm$ 0.51	7/12	7/12	0/1
ROka-gD2/E-infected HFF cells	12	12/12	4.08 $\pm$ 0.58 ^e	7.15 $\pm$ 0.29	0/12 ^f	1/12	8/8

^a Number of animals with clinical disease/number of animals with confirmed HSV-2 infection.

^b Means $\pm$ standard errors of the means measured as the area under the lesion score-day curve. Only symptomatic animals were included in the calculations.

^c Mean $\pm$ standard errors of the means (log₁₀) vaginal viral titers on 1st day following intravaginal HSV-2 challenge.

^d Number of infected animals that experienced recurrences/number of infected animals for which recurrences could be evaluated. Animals with significant bacterial superinfection were excluded.

^e $P = < 0.001$ (significance calculated by one-way analysis of variance with the Bonferroni correction for multiple groups).

^f $P = < 0.005$ (significance calculated by Fisher's exact test).

HSV-2 glycoproteins (25, 26). Improved protection against HSV-2 might be achieved by increasing the level of gD2 expression through the use of a stronger promoter. In addition, a VZV recombinant vaccine expressing other HSV-2 glycoproteins, such as HSV-2 gB, in addition to HSV-2 gD may be more immunogenic than the ROka-gD2 recombinant vaccine used in the current study. Combinations of HSV glycoproteins have been shown to provide protection superior to that of HSV gD alone when they are used as subunit vaccines (25). It should also be noted that VZV replication and disease are limited in guinea pigs (22). A live-VZV recombinant vaccine might replicate better in humans, the natural host of VZV, resulting in increased expression of foreign antigens and the development of immunity more protective than that achieved in guinea pigs.

VZV infects many fewer species of animals and cultured cell types than does HSV. HSV-gD participates in viral attachment and penetration (11, 17); therefore, the lack of a gD homolog in VZV may contribute to the narrow host range of VZV. However, infection of cells normally not permissive for VZV infection (HEp-2 cells) or poorly permissive for VZV infection (HeLa and Vero cells) with ROka-gD2 did not result in enhanced infection in vitro (14). It is unknown whether VZV recombinants expressing additional HSV glycoproteins may have expanded tissue tropism in humans.

VZV strain Oka is the only live DNA virus vaccine shown to be safe and effective in immunocompetent and immunocompromised people. Whereas two previous studies have demonstrated that VZV Oka can express exogenous proteins (18, 23), we have shown for the first time that a foreign antigen expressed in VZV Oka can be used as a vaccine to reduce the severity of disease in animals. Therefore, VZV Oka could serve as a useful vector for the expression of foreign proteins which may confer protective immunity in humans to other pathogens as well as to VZV.

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REFERENCES

- Arvin, A. M., S. Solem, C. Koropchak, E. Kinney-Thomas, and S. Paryani. 1987. Humoral and cellular immunity to varicella-zoster virus glycoprotein gp1 and to a non-glycosylated protein, p170, in the strain 2 guinea pig. *J. Gen. Virol.* **68**:2449–2454.
- Berman, P. W., T. Gregory, D. Crase, and L. A. Lasky. 1985. Protection from genital herpes simplex virus type 2 infection by vaccination with cloned type 1 glycoprotein D. *Science* **227**:1490–1492.
- Bernstein, D. I., L. R. Stanberry, C. J. Harrison, J. C. Kappes, and M. G. Myers. 1986. Antibody response, recurrent patterns, and subsequent herpes simplex virus type 2 (HSV-2) reinfection following initial HSV-2 infection of guinea pigs: effects of acyclovir. *J. Gen. Virol.* **67**:1601–1612.
- Campadelli-Fiume, G., and F. Serafini-Cessi. 1985. Processing of the oligosaccharide chains of herpes simplex virus type 1 glycoproteins, p. 357–382. *In* B. Roizman (ed.), *The herpesviruses*. Plenum Press, New York.
- Cohen, G. H., M. Katze, C. Hydrean-Stern, and R. J. Eisenberg. 1979. Type-common CP-1 antigen of herpes simplex virus is associated with a 59,000-molecular weight envelope glycoprotein. *J. Virol.* **27**:172–181.
- Cohen, J. I., and K. E. Seidel. 1993. Generation of varicella-zoster virus (VZV) and viral mutants from cosmid: VZV thymidylate synthetase is not essential for replication in vitro. *Proc. Natl. Acad. Sci. USA* **90**:7376–7380.
- Cohen, J. I., and K. E. Seidel. 1994. Absence of varicella-zoster virus (VZV) glycoprotein V does not alter growth of VZV in vitro or sensitivity to heparin. *J. Gen. Virol.* **75**:3087–3093.
- Cohen, J. I., and K. E. Seidel. 1994. Varicella-zoster virus (VZV) open reading frame 10 protein, the homolog of the essential herpes simplex virus protein VP16, is dispensable for VZV replication in vitro. *J. Virol.* **68**:7850–7858.
- Cohen, J. I., and K. E. Seidel. 1995. Varicella-zoster virus ORF1 encodes a membrane protein that is dispensable for growth of VZV in vitro. *Virology* **206**:835–842.
- Davison, A. J., and J. E. Scott. 1986. The complete DNA sequence of varicella-zoster virus. *J. Gen. Virol.* **67**:1759–1816.
- Fuller, A. O., and W. L. Lee. 1992. Herpes simplex virus type 1 entry through a cascade of virus-cell interactions requires different roles of gD and gH in penetration. *J. Virol.* **66**:5002–5012.
- Heineman, T. C., and J. I. Cohen. 1994. Deletion of the varicella-zoster virus large subunit of ribonucleotide reductase impairs growth of virus in vitro. *J. Virol.* **68**:3317–3323.
- Heineman, T. C., and J. I. Cohen. 1995. The varicella-zoster virus (VZV) open reading frame 47 (ORF47) protein kinase is dispensable for viral replication and is not required for phosphorylation of ORF63 protein, the VZV homolog of herpes simplex virus ICP22. *J. Virol.* **69**:7368–7371.
- Heineman, T. C., and J. I. Cohen. Unpublished data.
- Johnson, R. M., D. W. Lancki, F. W. Fitch, and P. G. Spear. 1990. Herpes simplex virus glycoprotein D is recognized as antigen by CD4+ and CD8+ T lymphocytes from infected mice: characterization of T cell clones. *J. Immunol.* **145**:702–710.
- Kohl, S., N. C. Strynadka, R. S. Hodges, and L. Pereira. 1990. Analysis of the role of antibody-dependent cellular cytotoxic antibody activity in murine neonatal herpes simplex virus infection with antibodies to synthetic peptides of glycoprotein D and monoclonal antibodies to glycoprotein B. *J. Clin. Invest.* **86**:273–278.
- Ligas, M. W., and D. C. Johnson. 1988. A herpesvirus mutant in which glycoprotein D sequences are replaced by β -galactosidase sequences binds to but is unable to penetrate cells. *J. Virol.* **62**:1486–1494.
- Lowe, R. S., P. M. Keller, B. J. Keech, A. J. Davison, Y. Whang, A. J. Morgan, E. Kieff, and R. W. Ellis. 1987. Varicella-zoster virus as a live vector for the expression of foreign genes. *Proc. Natl. Acad. Sci. USA* **84**:3896–3900.
- McGeoch, D. J., H. W. Moss, D. McNab, and M. C. Frame. 1987. DNA sequence and genetic content of the HindIII 1 region in the short unique component of the herpes simplex virus type 2 genome: identification of the gene encoding glycoprotein G, and evolutionary comparisons. *J. Gen. Virol.* **68**:19–38.
- Muggeridge, M. I., S. R. Roberts, V. J. Isola, G. H. Cohen, and R. J. Eisenberg. 1990. Herpes simplex virus, p. 235–241. *In* M. V. H. Van Regenmortel and A. R. Neurath (ed.), *Immunocytochemistry of viruses II: the basis for serodiagnosis and vaccines*. Elsevier, Amsterdam.
- Myers, M. G., B. Connelly, and L. Stanberry. 1991. Varicella in hairless guinea pigs. *J. Infect. Dis.* **163**:746–751.
- Myers, M. G., and B. L. Connelly. 1992. Animal models of varicella. *J. Infect. Dis.* **166** (Suppl. 1):S48–S50.
- Shiraki, K., Y. Hayakawa, H. Mori, J. Namazue, A. Takamizawa, I. Yoshida, K. Yamanishi, and M. Takahashi. 1991. Development of immunogenic recombinant Oka varicella vaccine expressing hepatitis B virus surface antigen. *J. Gen. Virol.* **72**:1393–1399.
- Stanberry, L. R. 1990. Subunit viral vaccines: prophylactic and therapeutic use, p. 309–341. *In* L. Aurelian (ed.), *Herpesviruses, the immune system and AIDS*. Kluwer Academic, Norwell.
- Stanberry, L. R., D. I. Bernstein, R. L. Burke, C. Pachl, and M. G. Myers. 1987. Vaccination with recombinant herpes simplex virus glycoproteins: protection against initial and recurrent genital herpes. *J. Infect. Dis.* **155**:914–920.
- Stanberry, L. R., R. L. Burke, and M. G. Myers. 1988. Herpes simplex virus glycoprotein treatment of recurrent genital herpes. *J. Infect. Dis.* **157**:156–163.
- Stanberry, L. R., E. R. Kern, J. T. Richard, T. M. Abbott, and J. C. Overall, Jr. 1982. Genital herpes in guinea pigs: pathogenesis of the primary infection and description of recurrent disease. *J. Infect. Dis.* **146**:397–404.
- Straus, S. E., L. Corey, R. L. Burke, B. Savarese, G. Barnum, P. R. Krause, R. G. Kost, J. L. Meier, R. Sekulovich, S. F. Adair, and C. L. Dekker. 1994. Placebo-controlled trial of vaccination with recombinant glycoprotein D of herpes simplex virus type 2 for immunotherapy of genital herpes. *Lancet* **343**:1460–1463.
- Straus, S. E., B. Savarese, M. Tigges, A. G. Freifeld, P. R. Krause, D. M. Margolis, J. L. Meier, D. P. Paar, S. F. Adair, D. Dina, C. L. Dekker, and R. L. Burke. 1993. Induction and enhancement of immune responses to herpes simplex virus type 2 in humans by use of a recombinant glycoprotein D vaccine. *J. Infect. Dis.* **167**:1045–1052.
- Takahashi, M. 1992. Current status and prospects of live varicella vaccine. *Vaccine* **10**:1007–1014.
- Wachsmann, M., L. Aurelian, C. C. Smith, B. R. Lipinskas, M. E. Perkins, and E. Paoletti. 1987. Protection of guinea pigs from primary and recurrent herpes simplex virus (HSV) type 2 cutaneous disease with vaccinia virus recombinants expressing HSV glycoprotein D. *J. Infect. Dis.* **155**:1188–1197.
- Watson, R. J. 1983. DNA sequence of the herpes simplex virus type 2 glycoprotein D gene. *Gene* **26**:307–312.