Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 7462–7466, October 1986
Medical Sciences

Expression of the F glycoprotein of respiratory syncytial virus by a recombinant vaccinia virus: Comparison of the individual contributions of the F and G glycoproteins to host immunity

(paramyxovirus/expression vector/fusion glycoprotein/neutralizing antibody/protective immunity)

ROBERT A. OLMSTED*, NARAYANASAMY ELANGO†‡, GREGORY A. PRINCE*, BRIAN R. MURPHY*, PHILIP R. JOHNSON*, BERNARD MOSS†, ROBERT M. CHANOCK*, AND PETER L. COLLINS*

*Laboratory of Infectious Diseases and †Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Contributed by Robert M. Chanock, May 29, 1986

ABSTRACT **A cDNA clone representing the mRNA coding sequence of the fusion glycoprotein (F) gene of human respiratory syncytial virus (RSV) was constructed and inserted into the thymidine kinase gene of vaccinia virus (WR strain) under the control of a vaccinia virus promoter. The resulting recombinant vaccinia virus, vaccinia F, expressed the F_1 and F_2 cleavage products (48 and 20 kDa, respectively) of the F glycoprotein in cell culture. F_1 and F_2 were indistinguishable from their authentic RSV counterparts with respect to glycosylation, disulfide linkage, electrophoretic mobility, cell-surface expression, and antigenic specificity. Cotton rats infected intradermally with vaccinia F developed a high titer of serum F-specific antibodies, which neutralized infectivity of RSV. This neutralizing antibody response exceeded that induced by infection of the respiratory tract with RSV and was 6-fold higher than that induced by vaccinia G, a recombinant vaccinia virus that expressed the RSV G glycoprotein gene. Immunization with vaccinia F stimulated almost complete resistance to replication of RSV in the lower respiratory tract as well as significant resistance in the upper respiratory tract. The degree of resistance conferred by vaccinia F exceeded that induced by vaccinia G.**

Human respiratory syncytial virus (RSV), a member of the pneumovirus genus of the family Paramyxoviridae, is the major etiologic agent of serious viral lower respiratory tract illness in infants and young children throughout the world (1). Previous attempts to develop a safe and effective RSV vaccine have all met with failure. A formalin-inactivated virus vaccine tested 20 years ago failed to protect against RSV infection or disease (2, 3). Instead, disease was enhanced during subsequent infection by RSV. Safe and immunogenic temperature-sensitive (ts) mutants of RSV were also developed but could not be used as a live vaccine because of overattenuation or genetic instability, which allowed the mutants to lose their ts phenotype (4). Immunization by parenteral inoculation of live RSV has also been evaluated as a strategy for prevention of disease, but a field trial indicated this approach to be ineffective (5).

Recent molecular studies of RSV, together with the development of techniques for producing live recombinant vaccinia virus (VV) vectors, offer new approaches to RSV immunoprophylaxis. For example, the two envelope glycoproteins of RSV have been identified as the major viral antigens responsible for inducing protective antibodies, and the genes for these two glycoproteins have been cloned and sequenced (6–9). The fusion glycoprotein F (70 kDa) mediates both viral penetration and cell–cell spread via membrane fusion (10). The F protein is synthesized as an inactive precursor, F_0, which is activated by proteolytic cleavage into two disulfide-linked polypeptide subunits, F_1 and F_2 (48 and 20 kDa, respectively) (11, 12). The major glycoprotein G (84–90 kDa) appears to be the viral attachment protein (13). Passive immunization studies in mice and cotton rats indicate that certain monoclonal antibodies (mAb) to either the F or G glycoprotein are able to protect the lower respiratory tract against infection with RSV (14, 15). However, the role of F and G in eliciting a protective immune response in the host remains largely undefined.

Recently, a full-length cDNA copy of the coding region of the RSV G glycoprotein was inserted by homologous recombination into the thymidine kinase (*TK*) gene of VV and the resulting recombinant VV, vaccinia G, was shown to express authentic G glycoprotein as indicated by glycosylation, cell-surface expression, and antigenic specificity (16, 17). Cotton rats infected with vaccinia G developed a high level of serum G-specific antibodies, which neutralized RSV, and these animals exhibited significant resistance in their lungs to replication of RSV after subsequent intranasal (i.n.) challenge with the virus. We now have constructed a live recombinant VV, vaccinia F, that expresses the F glycoprotein of RSV. Here we describe (*i*) the functional expression of F glycoprotein by vaccinia F in cell culture, (*ii*) the development of a high level serum antibody response to the F glycoprotein by cotton rats infected with vaccinia F, (*iii*) the ability of the vaccinia F to induce significant resistance to replication of RSV in the upper respiratory tract of cotton rats and almost complete resistance in the lower respiratory tract, and (*iv*) evidence that vaccinia F induces more effective immunity in the cotton rat than the previously studied vaccinia G.

MATERIALS AND METHODS

Cells and Viruses. Purified stocks of the parental VV (WR strain) and recombinant VV were prepared from infected HeLa cell suspension cultures as described (17). RSV (human strain A2) was plaque-purified three times and grown in HEp-2 cell monolayers. Recombinant VV vRSVG and vSC-8 have been described (17, 18) and are designated vaccinia G and vaccinia vSC-8, respectively.

Construction of the F cDNA. Following the strategy outlined in Fig. 1, 5 μg of the oligonucleotide 5′ CAC*GGATC*-*CTATTTA*GTTACTAAATGCAA (primer sequences repre-

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: RSV, respiratory syncytial virus; VV, vaccinia virus; mAb, monoclonal antibody(ies); i.n., intranasal; i.d., intradermal; TK, thymidine kinase; pfu, plaque-forming unit(s).

‡Present address: National Institute of Virology, 20A Dr. Ambedkar Road, Pune 411001, India.

senting a flanking *Bam*HI site and the complement of the translational–termination codon in the F mRNA are italicized) was used to direct reverse transcription of F mRNA in 80 μg of infected cell mRNA in a 0.4-ml reaction mixture containing actinomycin D (80 μg/ml) and 300 units of reverse transcriptase. Nucleic acids were purified with phenol/chloroform, and mRNA was hydrolyzed with NaOH (0.3 M, 37°C, 2 hr). The cDNA was purified by several cycles of ethanol precipitation, hybridized with 2.5 μg of the primer 5′ CAC*GGATCC*ACA*ATG*GAGTTGCTAATCCT (the flanking *Bam*HI site and translational–initiation codon of the F mRNA are italicized), and copied with reverse transcriptase in a 0.2-ml reaction mixture. The cDNAs (1 μg) were electrophoresed on a 1.4% low melting point agarose gel, and full-length F cDNA was visualized by staining with ethidium bromide, cut from the gel, and recovered from the molten gel slice by chromatography on a NACS column (Bethesda Research Laboratories). The cDNA (180 ng) was made blunt-ended by incubation with 3 units of T4 DNA polymerase at 37°C for 5 min in the absence of deoxynucleotides and 30 min in the presence of 0.25 mM of each deoxynucleotide. The cDNA was digested with *Bam*HI and cloned in pBR322, yielding 6–10 approximately full-length cloned inserts per ng of input cDNA. The details of these procedures followed published methods (19, 20).

Nucleotide Sequence Analysis. The sequence of the F cDNA in pBR322 was confirmed by dideoxynucleotide sequencing using the alkaline-denaturation method (21) and the following oligonucleotide primers, designated by specificity and sequence position: F-217, GTGTAATGGAACAGA; F-448, ATCTGCAATCGCCAG; F-700, ACTACTAGAGATTAC; F-952, TTGGAAACTACACAC; F-1213, AGATGTAAGCAGCTC; F-1444, CTATGACCCATTAGT; pBR322-333 (clockwise), CCACTATCGACTAC; pBR322-418 (counterclockwise), GGCGCCGGTGATGC. Oligonucleotides were synthesized on an Applied Biosystems (Foster City, CA) synthesizer.

Immunoprecipitation. Labeling of RSV structural proteins with [^{3}H]glucosamine was carried out in RSV-infected HEp-2 cells as described (7). CV-1 cells were infected with vaccinia F or vaccinia vSC-8 [30 plaque-forming units (pfu) per cell] for 2 hr. The inocula were removed and the cells were incubated with 300 μCi of [^{3}H]glucosamine per ml (1 Ci = 37 GBq) in Eagle's minimal essential medium supplemented with 2.5% fetal bovine serum for a period of 24 hr. Infected cell lysates were prepared (22) and clarified as described (23). Immunoprecipitations were carried out using mAb B-151 (anti-RSV-F) or B-158 (anti-RSV-G) (24) and protein A-Sepharose (23). Samples were boiled in electrophoresis sample buffer with or without 2-mercaptoethanol and analyzed by 10% NaDodSO$_4$/PAGE (22).

Immunological Response to Immunization and Protective Efficacy. Young adult cotton rats (*Sigmodon hispidus*) were immunized by intradermal (i.d.) inoculation with vaccinia F, vaccinia G, vaccinia vSC-8 (10^8 pfu) or by i.n. inoculation with RSV (10^4 pfu); 21 days after immunization, animals were bled and then challenged with RSV i.n. ($10^{5.3}$ pfu). Animals were sacrificed 4 days after challenge. F- and G-specific antibody responses were measured by ELISA using purified RSV F and G glycoproteins as antigens (10, 13, 25). RSV serum neutralizing antibody titers were determined by 60% plaque reduction neutralization assay. RSV titers in the nasal turbinates and lung homogenates were measured by plaque titration as described (25).

RESULTS

Construction of Vaccinia F. The nucleotide sequence of the RSV F mRNA has been determined from incomplete overlapping cDNAs (6, 7). To construct a full-length cDNA suitable for expression, F mRNA isolated from RSV-infected cells was transcribed into cDNA by the use of specific oligonucleotide primers that contained flanking *Bam*HI recognition sequences (Fig. 1; see *Materials and Methods*). This strategy was designed to generate F cDNAs that contain the entire coding frame flanked on each side by three additional nucleotides and a *Bam*HI restriction site. Many clones of the appropriate size were obtained; one clone, designated F11, whose sequence was confirmed in its entirety by dideoxynucleotide sequencing using synthetic oligonucleotides, was selected for further study. Its sequence differed from that of Collins *et al.* (6) at four positions (nucleotide 198 was C, Met→Thr; 212 was G, Pro→Ala; 1148 was G, Ile→Val; 1352 was G, Met→Val) and differed from that of Elango *et al.* (7) at one position (198 was C, Met→Thr). Nucleotide variability at these positions in different cDNA clones was described by Collins *et al.* and probably represents naturally occurring viable variants in the virus population (6).

The F cDNA was inserted into the VV plasmid coexpres-

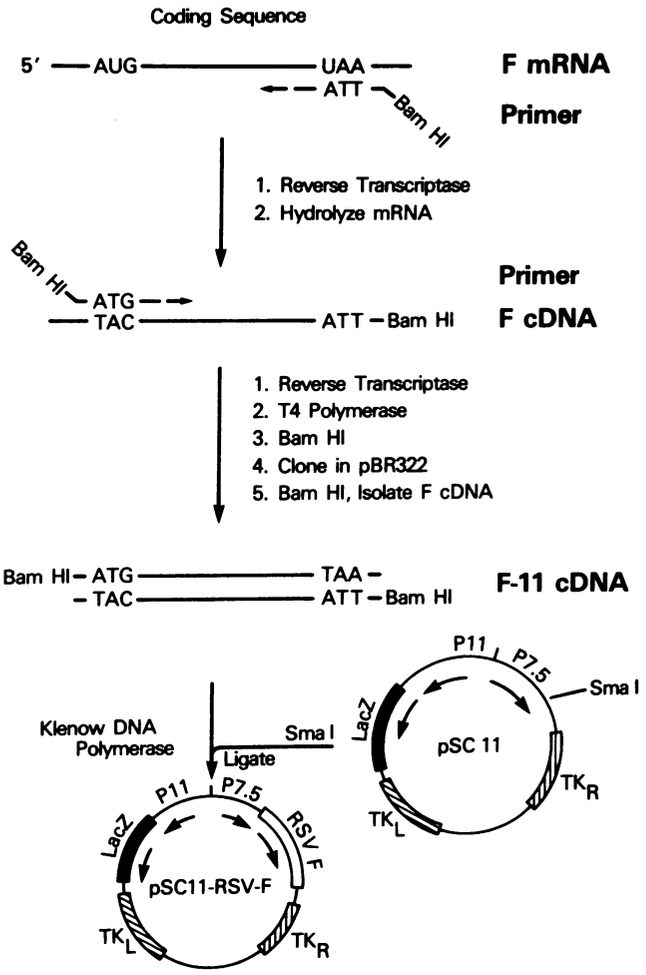

FIG. 1. Construction of a cDNA of the complete F glycoprotein coding sequence under the control of a VV promoter. A 29-nucleotide oligonucleotide primer that spanned the termination codon of the F mRNA and contained a flanking *Bam*HI recognition site was hybridized to F mRNA and used to direct cDNA synthesis. A second 29-nucleotide primer that spanned the initiator codon of the F mRNA and contained a flanking *Bam*HI site was used to direct synthesis of the second cDNA strand. Additional steps in the cloning, isolation, and sequencing of the F cDNA are outlined above and in *Materials and Methods*. The F cDNA from one recombinant, F-11, was isolated and inserted into the *Sma* I site of the coexpression vector pSC11 to generate pSC11-RSV-F. This vector was subsequently used to direct the insertion, by homologous recombination, of the F cDNA construct into the VV genome.

sion vector pSC11 (Fig. 1). The features of this construct include (*i*) the F cDNA is under the control of the early-late VV promoter P7.5; (*ii*) the vector contains the *Escherichia coli* β-galactosidase gene under the control of the late VV promoter, P11; and (*iii*) both chimeric genes are flanked by VV TK sequences. Upon transfection of VV-infected cells, the TK sequences direct homologous recombination into the TK locus of the VV genome, yielding live recombinant VV that are identified colorimetrically by the presence of β-galactosidase activity. Methods for obtaining such recombinants have been described in detail (17, 18).

Expression of the F Glycoprotein by Vaccinia F *in Vitro*. To confirm the functional expression of F by vaccinia F, recombinant virus-infected CV-1 cells were labeled with [^{3}H]glucosamine and immunoprecipitates of cell lysates were analyzed by PAGE in the presence or absence of 2-mercaptoethanol (Fig. 2). The results of this experiment demonstrated that vaccinia F directed the expression of polypeptides that were indistinguishable from F_1 and F_2 glycoproteins produced in RSV-infected HEp-2 cells with respect to electrophoretic mobility and glycosylation. PAGE analysis of these immune complexes under nonreducing conditions confirmed that the F_1 and F_2 glycoproteins expressed by vaccinia F are linked by disulfide bonds and on this basis are indistinguishable from authentic $F_{1,2}$. Immunoprecipitation of these proteins by the anti-F mAb provided further proof of their authenticity and antigenic specificity. These proteins were not immunoprecipitated from vaccinia F lysates by the anti-G mAb or from infected cell lysates of vaccinia-vSC-8 (18), a TK$^-$ vaccinia recombinant expressing only β-galactosidase as the foreign gene, using anti-F mAb (Fig. 2).

The final stages of posttranslational processing of the F glycoprotein involve transport to and localization in the plasma membrane of the host cell. The cellular location of F glycoprotein expressed by vaccinia F was investigated by fluorescent antibody staining of formalin-fixed cells to detect antigens expressed at the cell surface, and acetone-fixed cells to detect intracellular antigen. The level of cell-surface fluorescence observed for vaccinia F-infected cells was comparable to that of RSV-infected cells, suggesting that the amounts of F present at the respective cell surfaces were similar (Fig. 3). The amounts of intracellular F antigen also appeared to be similar, and in both cases the staining was localized to the perinuclear region, consistent with localization at the rough endoplasmic reticulum and Golgi complex (not shown). Specific fluorescent staining was not observed with parental VV (WR strain)-infected cells (Fig. 3).

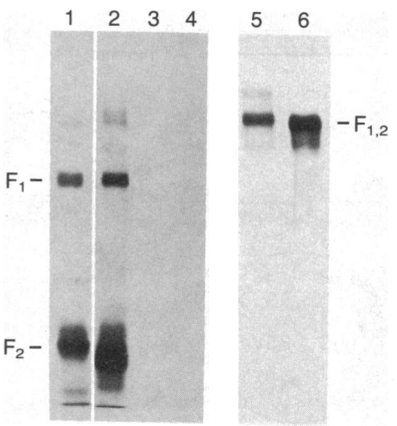

FIG. 2. Immunoprecipitation of F glycoprotein synthesized during infection by vaccinia F or RSV. CV-1 cells were infected with vaccinia F or with a recombinant VV control, vaccinia vSC-8 (see *Materials and Methods*) and were incubated with [^{3}H]glucosamine in parallel with RSV-infected HEp-2 cells. Clarified lysates were prepared and incubated with either anti-F mAb or anti-G mAb. Immunoprecipitates were analyzed by 10% PAGE under reducing (lanes 1–4) and nonreducing (lanes 5 and 6) conditions. Lanes: 1 and 6, RSV cell lysates incubated with anti-F mAb; 2 and 5, vaccinia F cell lysates incubated with anti-F mAb; 3, vaccinia F cell lysates incubated with anti-G mAb; 4, vaccinia vSC-8 cell lysates incubated with anti-F mAb. A fluorogram of the dried gel is shown with the positions of the disulfide-linked ($F_{1,2}$) and monomeric (F_1 and F_2) subunits marked.

Serologic Response to Infection by Vaccinia F. Cotton rats were inoculated i.d. with vaccinia F (10^8 pfu) to determine its ability to induce serum antibodies and resistance to subsequent RSV infection. For comparison, vaccinia G was included in this study to evaluate in parallel the relative importance of the two RSV glycoproteins in inducing host immunity. At 21 days postvaccination, serum samples were collected and assayed for RSV antibodies by F- and G-specific ELISA and plaque reduction neutralization assay (Table 1). The immune response to the recombinants was antigen specific because animals infected with the vaccinia F recombinant developed a significant antibody response only to F and the vaccinia G-infected animals developed a significant response only to G. As shown previously (17), cotton rats infected with RSV developed serum ELISA antibodies for both glycoproteins. Significantly, the titers of ELISA antibodies to the RSV glycoproteins that developed in animals infected with vaccinia F or vaccinia G were 5- to 6-fold higher than those observed in animals infected with RSV i.n. (Table 1). Control values for this assay are represented by the levels of F and G antibodies measured in the sera of unimmunized cotton rats or cotton rats immunized with vaccinia vSC-8. It should be noted that a low level of antibodies cross-reactive with the F glycoprotein appeared to be induced by vaccinia vSC-8, but this effect has not been further characterized.

Infection by vaccinia F also induced high levels of serum-neutralizing antibodies to RSV that were 2- to 3-fold higher in titer than those induced by infection of the respiratory tract by RSV (Table 1). The serum-neutralizing antibody response to vaccinia F was 13-fold greater than in animals infected with live RSV i.d. (not shown). In the present comparative study, the mean serum-neutralizing antibody titer induced by vaccinia G was 2-fold higher than previously reported (17). However, the serum-neutralizing antibody titer induced by vaccinia G in the present study was one-sixth that stimulated

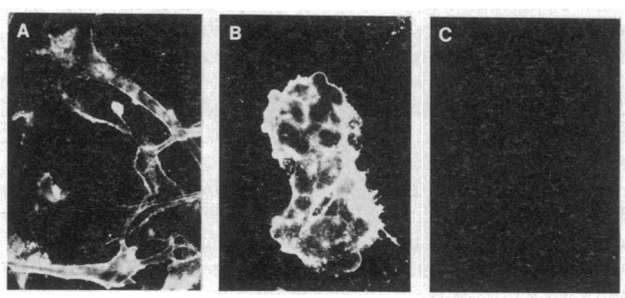

FIG. 3. Characterization of F glycoprotein expression by cell surface immunofluorescence. CV-1 cells grown on glass coverslips were infected with 1 pfu per cell of RSV (*A*), vaccinia F (*B*), and parental VV (WR strain) (*C*). The cells were incubated for 28 hr, fixed with 3% formalin in phosphate-buffered saline (PBS) for 15 min at room temperature, and dried. Fixed cells were incubated for 30 min at 37°C with a rabbit monospecific polyclonal anti-RSV-F serum, which was prepared against immunoaffinity purified F glycoprotein (10). The cells were washed three times with PBS and incubated with a goat anti-rabbit IgG fluorescein-conjugated antibody. Antibody dilutions were made in PBS/1% ovalbumin. After 30 min at 37°C, the cells were washed five times with PBS, dipped in H_2O to remove salts, dried, and mounted with PBS/50% glycerol. Immunofluorescence was observed and photographed with a Leitz-Dialux fluorescent microscope.

Table 1. Immunogenicity and protective efficacy of vaccinia RSV F and G glycoprotein recombinants in cotton rats

Immunization	pfu	Serologic response 21 days postimmunization: No. of animals	ELISA (log_2)*: F	ELISA (log_2)*: G	Serum neutralizing antibody (log_2)†	Response to RSV i.n. challenge ($10^{5.3}$ pfu) 21 days postimmunization: No. of animals	Titer of virus recovered, log_{10} pfu/g‡: Nose	Titer of virus recovered, log_{10} pfu/g‡: Lungs
None		18	5.7	6.0	4.3§	18	6.1 ± 0.1	6.2 ± 0.1
RSV (i.n.)	$10^{4.0}$	14	15.8	11.8	10.7	5	<2.0¶	<2.0¶
Vaccinia F (i.d.)	$10^{8.0}$	22	18.4	7.3	12.2	19	3.7 ± 0.3¶	2.2 ± 0.1¶‖
Vaccinia G (i.d.)	$10^{8.0}$	23	7.3	14.2	9.6	6	6.1 ± 0.1	3.5 ± 0.5¶‖
Vaccinia F + vaccinia G (i.d.)	$10^{8.0}$	13	18.1	15.6	12.2	11	4.0 ± 0.3¶	<2.0¶
Vaccinia vSC-8 (i.d.) (β-galactosidase, TK^-)	$10^{8.0}$	15	8.6	6.6	5.2	7	6.1 ± 0.1	6.1 ± 0.1

*Mean serum antibody titer (reciprocal, log_2) using purified RSV F or G glycoprotein as antigen.
†Mean serum antibody titer (reciprocal, log_2) measured by 60% plaque reduction neutralization of RSV.
‡Mean titer ± SEM. Minimum level of detectability was $10^{2.0}$.
§Only 12 were tested for neutralizing antibody.
¶$P < 0.001$ compared to control groups by t test.
‖$P < 0.001$ by t test.

by vaccinia F. When the vaccinia F and vaccinia G recombinants were administered together, there was no increase in serum ELISA or neutralizing antibody titer over that induced by vaccinia F alone (Table 1).

Protective Efficacy of Vaccinia F. At 21 days postimmunization, the cotton rats were challenged with $10^{5.3}$ pfu of RSV inoculated i.n. Four days later, the animals were sacrificed and the levels of virus replication in the nose and lungs were measured by plaque assay. Control animals, which were unimmunized or immunized with vaccinia vSC-8, supported a high level of virus replication in their upper and lower respiratory tract (Table 1). In contrast, the lower respiratory tract of cotton rats previously infected i.d. with vaccinia F exhibited almost complete resistance to RSV, a level of immunity essentially equivalent to that induced by RSV infection of the respiratory tract. Also, prior infection by vaccinia F induced significant resistance to virus replication in the nose. However, this resistance was demonstrably less than that observed after RSV infection (Table 1). These data clearly indicate that the upper and lower respiratory tract of cotton rats immunized with vaccinia F exhibit significant resistance to challenge with a large dose of RSV ($10^{5.3}$ pfu). It should be noted that vaccinia G induced significantly less resistance to RSV in the lower respiratory tract than did vaccinia F. Also, vaccinia G did not induce demonstrable resistance in the upper respiratory tract.

DISCUSSION

We have described the construction of a cDNA copy of the RSV F glycoprotein coding sequence, its insertion into the *TK* gene of VV, and its expression in cell culture and experimental animals. F synthesized during infection by vaccinia F could not be distinguished from F produced in RSV-infected cells with respect to proteolytic processing, glycosylation, disulfide linkage, electrophoretic migration, cell-surface expression, or antigenic specificity. However, F-mediated cell-membrane fusion was not detected unequivocally in vaccinia F-infected cell cultures, probably because of the rapid and extensive cytopathic effects caused by VV infection. To confirm this functional activity of the vector-encoded F glycoprotein, we analyzed the expression of the same F cDNA under the control of the simian virus 40 late promoter in the recombinant simian virus 40 vector SV2330 (26). The kinetics of proteolytic processing of F expressed by the simian virus 40 vector were similar to those observed during RSV infection and, more importantly, F expressed at the cell surface by this vector induced widespread cell–cell fusion of monkey cell monolayers (R.A.O. and P.L.C., unpublished observations).

VV vectors expressing foreign viral genes permit a direct evaluation of the relative contribution of individually expressed proteins or glycoproteins to host immunity. For example, infection of cotton rats by vaccinia G recently was shown to induce a high level of serum G-specific antibodies that neutralized infectivity of RSV (17). In the current study, we extended our analysis of RSV protective antigens by comparing the immunological response of cotton rats to infection by vaccinia F or vaccinia G. Both vaccinia F and vaccinia G stimulated higher levels of F- or G-specific serum antibodies (as measured by ELISA) than RSV infection of the respiratory tract. A higher titer of serum-neutralizing antibodies was also induced by the vaccinia F recombinant. Significantly, the vaccinia F recombinant stimulated a 6-fold higher level of serum-neutralizing antibodies than the vaccinia G recombinant. Nonetheless, both recombinants induced levels of neutralizing antibodies that exceed the level required to protect the lungs of cotton rats against RSV infection (27). The neutralizing antibody titers induced by vaccinia F or vaccinia G were also higher than the mean titer of maternally derived antibodies present in the serum of young infants <2 months of age who exhibit a relative resistance to severe RSV disease (28).

A single i.d. inoculation of cotton rats with vaccinia F induced almost complete resistance to lower respiratory tract infection by RSV. Significant but not complete resistance to upper respiratory tract infection was also induced. The ability of vaccinia G to induce resistance in the lungs was confirmed, but the level of protection observed was less than that described (17). Unlike the previous study involving vaccinia G, resistance was not observed in the upper respiratory tract. The smaller protective effect of vaccinia G in the present study can be explained by the higher dose of RSV used for challenge, $10^{5.3}$ pfu rather than $10^{4.0}$ pfu. Replication of RSV in the nose and lung tissues of the control animals in the present study was significantly higher than in the previous study, and this permitted us to detect a greater protective effect of immunization as well as to distinguish differences in protective efficacy of the F and G recombinants. It is likely that the higher titer of serum-neutralizing antibodies induced by vaccinia F accounted, in part, for its greater protective effect compared to vaccinia G.

Failure of the high level of serum-neutralizing antibodies induced by vaccinia F to completely protect the nose was not unexpected, because a previous study of passive immunoprophylaxis indicated that a high titer of serum-neutralizing antibodies provided only partial protection in the upper respiratory tract, whereas the lungs were completely protected (27). Complete resistance in the nose to RSV infection can only be induced by infection at this site, suggesting that local immunity is the main mechanism responsible for resistance to this virus in the upper respiratory tract. Nevertheless, a form of immunization such as vaccinia F, which almost completely protects the lungs but leaves the nose partially permissive for RSV, may be advantageous. A moderate level of virus replication in the upper respiratory tract during subsequent infection would be expected to boost local and systemic immunity under conditions in which serious lower respiratory tract disease did not occur.

In young infants, maternally derived RSV antibodies exert a suppressive effect on the immune response to the RSV G glycoprotein (B.R.M., unpublished data). This form of immunosuppression also may adversely affect the young infants' response to RSV glycoproteins expressed by VV recombinants. Attempts to demonstrate glycoproteins of other enveloped viruses on the surface of recombinant VV have not been successful (G. Kotwal, personal communication) and by analogy it seems unlikely that pre-existing RSV serum antibodies will inhibit the replication of the F and G vaccinia recombinants *in vivo*.

Twenty years ago a formalin-inactivated RSV vaccine was observed to potentiate disease in vaccinated children when they subsequently underwent natural RSV infection (2, 3). Prince *et al.* recently were able to reproduce the phenomenon of disease potentiation by vaccinating cotton rats with formalin-inactivated RSV (25). Formalin treatment of RSV appears to selectively destroy the protective neutralization epitopes of the RSV surface glycoproteins. Immunization with this material produced an unbalanced immune response in which the host developed high levels of F- and G-specific antibodies that lacked neutralizing activity. Thus, most of the antibodies induced by formalin-inactivated RSV vaccine were directed against nonprotective epitopes on the viral surface glycoproteins, and these antibodies were available to form immune complexes with viral antigens at the time of RSV infection. In the formulation of RSV vaccination strategies, it is essential to use an immunogen that will stimulate biologically functional (i.e., neutralizing) antibodies in high titer in order to induce resistance without risking potentiation of RSV disease. The vaccinia F and vaccinia G recombinants meet this requirement because they both stimulate a balanced immune response with respect to ELISA and neutralizing antibody levels. The ratios of these antibody titers induced by the vaccinia RSV F or G recombinant were similar to those observed following RSV infection. Thus, potentiation of disease by vaccinia F or G would seem unlikely.

We thank E. Camargo, R. L. Horswood, and N. Cooper for technical assistance; A. Buckler-White for the synthesis of oligonucleotides; S. Chakrabarti for the coexpression vector; E. Norrby for mAb B151 and B158; Praxis Biologic for rabbit monospecific anti-F serum; E. E. Walsh for purified RSV glycoproteins; and S. Chang, L. Jordan, and S. Harding for editorial assistance.

1. McIntosh, K. & Chanock, R. M. (1985) in *Virology*, ed. Fields, B. N. (Raven, New York), pp. 1285–1304.
2. Kim, H. W., Canchola, J. G., Brandt, C. D., Pyles, G., Chanock, R. M., Jensen, K. & Parrott, R. H. (1969) *Am. J. Epidemiol.* **89,** 422–434.
3. Kapikian, A. Z., Mitchell, R. H., Chanock, R. M., Shvedoff, R. A. & Stewart, C. E. (1969) *Am. J. Epidemiol.* **89,** 405–421.
4. Wright, P. F., Belshe, R. B., Kim, H. W., Van Voris, L. P. & Chanock, R. M. (1982) *Infect. Immun.* **37,** 397–400.
5. Buynak, E. B., Weibel, R. E., McLean, A. A. & Hilleman, M. R. (1978) *Proc. Soc. Exp. Biol. Med.* **157,** 636–642.
6. Collins, P. L., Huang, Y. T. & Wertz, G. W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7683–7687.
7. Elango, N., Satake, M., Coligan, J. E., Norrby, E., Camargo, E. & Venkatesan, D. (1985) *Nucleic Acids Res.* **13,** 1559–1574.
8. Wertz, G. W., Collins, P. L., Huang, Y., Gruber, C., Levine, S. & Ball, L. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4075–4079.
9. Satake, M., Coligan, J. E., Elango, N., Norrby, E. & Venkatesan, S. (1985) *Nucleic Acids Res.* **13,** 7795–7812.
10. Walsh, E. E., Brandiss, M. W. & Schlesinger, J. J. (1985) *J. Gen. Virol.* **66,** 409–415.
11. Fernie, B. F. & Gerin, J. L. (1982) *Infect. Immun.* **37,** 243–249.
12. Gruber, C. & Levine, S. (1983) *J. Gen. Virol.* **64,** 825–832.
13. Walsh, E. E., Schlesinger, J. J. & Brandiss, M. W. (1984) *J. Gen. Virol.* **65,** 761–767.
14. Taylor, G., Stott, E. J., Bew, M., Fernie, B. F., Cote, P. J., Collins, A. P., Hughes, M. & Jebbett, J. (1984) *Immunology* **52,** 137–142.
15. Walsh, E. E., Schlesinger, J. J., Brandiss, M. W. (1984) *Infect. Immun.* **43,** 756–758.
16. Ball, L. A., Young, K. K. Y., Anderson, K., Collins, P. L. & Wertz, G. W. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 246–250.
17. Elango, N., Prince, G. A., Murphy, B. R., Venkatesan, S., Chanock, R. M. & Moss, B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1906–1910.
18. Chakrabarti, S., Brechling, K. & Moss, B. (1985) *Mol. Cell Biol.* **5,** 3403–3409.
19. Collins, P. L. & Wertz, G. W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3208–3212.
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
21. Spriggs, M. K., Olmsted, R. A., Venkatesan, S., Coligan, J. E. & Collins, P. L. (1986) *Virology* **152,** 241–251.
22. Olmsted, R. A., Baric, R. S., Sawyer, B. A. & Johnston, R. E. (1984) *Science* **225,** 424–427.
23. Sveda, M. M., Markoff, L. J. & Lai, C.-J. (1982) *Cell* **30,** 649–656.
24. Mufson, M. A., Orvell, C., Rafnar, B. & Norrby, E. (1985) *J. Gen. Virol.* **66,** 2111–2124.
25. Prince, G. A., Jenson, A. B., Hemming, V. G., Murphy, B. R., Walsh, E. E., Horswood, R. L. & Chanock, R. M. (1986) *J. Virol.* **57,** 721–728.
26. Sveda, M. M. & Lai, C.-J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5488–5492.
27. Prince, G. A., Horswood, R. L. & Chanock, R. M. (1985) *J. Virol.* **55,** 517–520.
28. Parrott, R. H., Kim, H. W., Arrobio, J. O., Hodes, D. S., Murphy, B. R., Brandt, C. D., Camargo, E. & Chanock, R. M. (1973) *Am. J. Epidemiol.* **98,** 298–300.