Comparison of the Relative Roles of the F and HN Surface Glycoproteins of the Paramyxovirus Simian Virus 5 in Inducing Protective Immunity

REAY G. PATERSON,¹ ROBERT A. LAMB,^{1*} BERNARD MOSS,² AND BRIAN R. MURPHY³

Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois 60201,¹ and Laboratory of Viral Diseases² and Laboratory of Infectious Diseases,³ National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892

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To compare the relative roles of the paramyxovirus simian virus 5 (SV5) major surface glycoproteins, fusion (F) and hemagglutinin-neuraminidase (HN), in inducing protective immunity, two recombinant vaccinia viruses were constructed. The F and HN polypeptides expressed by the recombinant viruses were indistinguishable from their authentic SV5 counterparts in electrophoretic mobility, glycosylation, and, for the F protein, cleavage of the precursor, F₀, to the disulfide-linked subunits F₁ and F₂. Injection of rabbits and hamsters with live recombinant virus elicited an antibody response to either F or HN and provided a source of monospecific polyclonal antisera to the SV5 proteins. The vaccinia virus-SV5 F (vaccinia-F) recombinant induced higher levels of neutralizing antibody than did the vaccinia-HN recombinant, but animals inoculated with vaccinia-HN were better protected from challenge with SV5. Animals infected with both the vaccinia-HN and vaccinia-F viruses were nearly as well protected from challenge as were animals infected with SV5.

Simian virus 5 (SV5) is a prototype paramyxovirus that is associated with lower respiratory tract disease in dogs. The envelope of the SV5 virion contains two integral membrane glycoproteins, hemagglutinin-neuraminidase (HN) and fusion (F), which are the major antigenic determinants (2). Studies using purified preparations of biologically active F and HN have shown that the F protein is involved in virus penetration, cell fusion, and hemolysis, whereas HN has both hemagglutinating and neuraminidase activities (7, 28-30).

The properties of antibodies to F and HN of paramyxoviruses have been investigated extensively with some conflicting results, primarily concerning the ability of the F glycoprotein to induce neutralizing antibodies (6, 10, 13, 16-19, 21-23, 31, 33). In particular, most studies have found F antibodies to have little or no neutralizing activity. It has not been resolved whether these differences are due to incomplete purification of the antigens or inactivation of some epitopes, principally of the F protein, during biochemical manipulations. Both problems could be avoided by immunizing animals with live vaccinia viruses that express individual paramyxovirus proteins.

We have recently expressed biologically active F and HN proteins from SV5 cDNAs by using an SV40 late-region replacement vector (24, 25). In the study described here, we used the same cDNAs for construction of recombinant vaccinia viruses. The properties of antibodies to the individual F and HN proteins induced by infection of Golden Syrian hamsters with the recombinant vaccinia viruses and their role in protection against SV5 infection were investigated.

MATERIALS AND METHODS

Cells and viruses. Monolayer cultures of the TC7 clone of CV1 cells were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum. Thymidine kinase-

negative (TK⁻) 143 cells were grown as monolayer cultures in minimal essential medium supplemented with glutamine and 10% fetal calf serum containing 25 µg of 5-bromo-2'-deoxyuridine per ml. The parental vaccinia virus (WR strain) and the vaccinia recombinant viruses were grown in monolayers of CV1 cells, and purified stocks were prepared as described previously (8). The W3 strain of SV5 (1) was grown in MDBK cells, as described previously (26).

Plasmid construction, transfection, and isolation of recombinant viruses. SV5 F- and HN-specific cDNAs were excised from plasmids pSV103F and pSV103HN (25) and ligated into the *Xho*I site of the vaccinia virus insertion vector pMM34 (12). CV1 cells infected with wild-type (WT) vaccinia virus were transfected with the recombinant plasmids, as described previously (34). TK⁻ recombinant viruses were isolated by plaque assay in TK⁻ 143 cells in the presence of 25 µg of 5-bromo-2'-deoxyuridine per ml, and recombinants were identified by DNA dot blot hybridization (11) and immunoprecipitation of infected cells (9) by using anti-F or anti-HN immunoglobulin G (IgG) (13, 14). Vaccinia virus-SV5 F (vaccinia-F) and vaccinia-HN recombinant viruses were plaque purified twice, amplified in TK⁻ cells in the presence of 5-bromo-2'-deoxyuridine, and grown in CV1 monolayers.

Radioisotopic labeling of polypeptides in infected cells, immunoprecipitation, and polyacrylamide gel electrophoresis. Monolayers of CV1 cells were infected with the W3 strain of SV5 at a multiplicity of 10 PFU per cell and labeled with 100 µCi of [³⁵S]methionine per ml for 1.5 h, from 18 to 19.5 h postinfection (p.i.). CV1 cells were infected with 30 PFU of either WT vaccinia virus or the vaccinia-F or -HN recombinant per cell and labeled with 100 µCi of [³⁵S]methionine per ml for 1.5 h, between 3 and 4.5 h p.i. Labeled infected-cell lysates were immunoprecipitated as described previously (9) by using monospecific antisera to purified SV5 F and HN proteins (13, 14; kindly provided by D. C. Merz) and analyzed on 15% polyacrylamide gels (9). For tunicamycin treatment, the drug was added at 1 µg/ml to infected cells 2.5

* Corresponding author.

h before radioactive labeling. At the times described above, infected cells were labeled with 125 μ Ci of [35 S]methionine per ml for 1 h in the presence of tunicamycin, and immunoprecipitation and polyacrylamide gel electrophoresis were performed.

Immunization of rabbits. Female New Zealand White rabbits (Research Industries) received $10^{8.3}$ PFU of either the vaccinia-F or vaccinia-HN recombinant subcutaneously in 0.2 ml of inoculum distributed among three sites on the back (one animal per antigen). A second set of injections were administered 28 days after the first set. The animals were bled at weekly intervals after the first set of injections, and serum obtained on day 21 after the first injection was used in the studies described here.

Animal studies. Eight-week-old female Golden Syrian hamsters free of Sendai virus, SV5, and pneumonia virus of mice were obtained from the National Institutes of Health animal production facility. The animals were anesthetized with pentobarbital and received either $10^{5.0}$ 50% tissue culture infective doses (TCID₅₀s) of SV5 in 0.1 ml of inoculum administered intranasally or $10^{8.3}$ PFU of WT vaccinia virus, the vaccinia-F or vaccinia-HN recombinant, or the vaccinia-F recombinant plus the vaccinia-HN recombinant injected intradermally in 0.1 ml of inoculum. On days 0 and 28, blood was obtained from the retro-orbital plexus for serological assays. On day 28, each hamster (12 in each group) was challenged with $10^{5.0}$ TCID₅₀s of SV5 intranasally, and the lungs were removed 4 days later for determination of the level of virus replication. Lung homogenates (10%, wt/vol) were prepared (27), and the amount of virus present was determined by infectivity titration by using MDCK cell monolayers in six-well plates (Costar, Cambridge, Mass.), measuring the hemadsorbing endpoints 5 days after infection by using a 0.1% suspension of guinea pig erythrocytes. The titers are expressed as TCID₅₀s per gram; the mean titers for the 12 hamsters in each group were determined. The serum from each hamster was tested for ability to inhibit hemagglutination by the hemagglutination inhibition assay (4) with four agglutinating units of SV5 and guinea pig erythrocytes.

The SV5 specific antibody responses were measured by an enzyme-linked immunosorbent assay (ELISA) similar to that described previously (15) except that zonally purified SV5 was diluted to contain a hemagglutination titer of 8 for use as antigen to coat the Immulon I U-bottom plates. A goat anti-hamster immunoglobulin heavy- and light-chain antiserum conjugated with horseradish peroxidase (Cooper Biomedical, Inc., West Chester, Pa.) was used at a dilution of 1:5,000, incubated overnight, and developed with azino-di-3-ethyl-benzothiazoline-6-sulfuric acid, as described previously (32). SV5 serum-neutralizing antibody titers were determined by a 60% plaque reduction assay with MDCK cells in 12-well Costar plates. Virus was diluted, mixed with a 10% solution of guinea pig complement (MA Bioproducts, Walkersville, Md.) mixed 1:1 with a dilution of heat-inactivated (56°C for 30 min) hamster serum, and incubated for 1 h at room temperature. A 0.1-ml portion of the virus-serum mixture was added to each well (two wells per serum dilution) and incubated at room temperature for 1 h. Infected monolayers were washed twice before being overlaid with modified 2 $\times$ L-15 medium, containing HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; 1 M; pH 6.8) and 7% NaHCO₃, mixed 1:1 with 1.6% agarose and were incubated at 37°C for 4 days. The agarose overlay was removed, and plaques were visualized by the addition of a 0.1% suspension of guinea pig erythrocytes. Hamster sera

were further characterized by immunoprecipitation assays with [35 S]methionine-labeled SV5-infected-cell lysates as the antigen as described above.

RESULTS

Plasmid construction. cDNA clones containing the complete coding regions of the SV5 F and HN mRNAs have previously been sequenced (5, 24), and biologically active F and HN proteins have been expressed on the surface of cells by using an SV40 late-region expression vector (25). To express the SV5 glycoproteins in vaccinia virus, the F- and HN-specific cDNAs were excised from the SV40 recombinant plasmids and inserted at the *Xho*I site of the plasmid vector pMM34 (12), as described in Materials and Methods. In the pMM34 F and HN recombinant plasmids the inserted cDNAs are placed under the control of the vaccinia virus P7.5 promoter, which functions both early and late during vaccinia virus replication (3). The inserted genes are flanked by sequences derived from the vaccinia virus TK gene. Vaccinia virus recombinants containing the cDNAs were isolated as a result of homologous recombination occurring between the TK sequences in the pMM34 F and HN plasmids and the TK gene in WT vaccinia virus, as previously described (12).

Expression of F and HN proteins from vaccinia virus recombinants and immunogenicity of the vaccinia-F and -HN recombinants in rabbits. To demonstrate the expression of the F and HN proteins by the recombinant vaccinia viruses, CV1 cells were infected with the vaccinia-F and vaccinia-HN recombinants and labeled with [35 S]methionine, and infected-cell lysates were immunoprecipitated with either anti-F or anti-HN protein IgG (13). The vaccinia-F (Fig. 1A, lane 5) and vaccinia-HN (lane 10) viruses expressed proteins that were immunoprecipitated by anti-F and anti-HN protein IgG, respectively. The expressed proteins had electrophoretic mobilities indistinguishable from those of the F (lane 3) and HN (lane 8) proteins in SV5-infected cells. In addition, the F₀ protein expressed by the vaccinia-F virus (lane 5) was cleaved into the disulfide-linked subunits F₁ and F₂ similarly to SV5 F₀ (lane 3). To obtain further evidence for the correct expression of the glycoproteins, vaccinia-F and -HN recombinant virus-infected cells were treated with tunicamycin, an inhibitor of N-linked glycosylation. Vaccinia-F-specific (Fig. 1A, lane 7) and -HN-specific (lane 14) polypeptides were observed with mobilities identical to those of F (lane 6) and HN (lane 12) proteins from SV5-infected CV1 cells treated with tunicamycin.

The biological activity of the glycoproteins expressed at the surface of the vaccinia recombinant virus-infected cells was examined. Vaccinia-F-virus-infected CV1 cells were overlaid at 4 h p.i. with fusion indicator BHK-21F cells from a freshly trypsinized monolayer. Extensive syncytium formation was observed at 15 h p.i. similar to that found for F protein expressed by an SV40 vector (25; data not shown). Vaccinia-HN-virus-infected cells were capable of hemadsorbing chicken erythrocytes, as found for HN expressed by an SV40 vector (25; data not shown).

To test the ability of the vaccinia-F and -HN recombinant viruses to elicit an antibody response to the F and HN proteins, rabbits were immunized with purified preparations of vaccinia-F or vaccinia-HN viruses. The preimmune and immune sera from the rabbits were assayed for antibodies to the SV5 F and HN proteins by immunoprecipitation of SV5-infected-cell lysates. The vaccinia-F and -HN viruses were able to elicit antibodies which recognized the F and HN proteins from SV5-infected cells (Fig. 1B).

FIG. 1. F and HN polypeptides synthesized in vaccinia-F virus-, vaccinia-HN virus-, or SV5-infected cells and immunoprecipitated with anti-F protein IgG, anti-HN IgG, or antibodies raised to infectious vaccinia-F and vaccinia-HN recombinant viruses in rabbits. CV1 cells were infected with SV5 at 10 PFU per cell and labeled with 100 μ Ci of [35 S]methionine per ml for 1.5 h at 18 h p.i. CV1 cells were infected with 30 PFU of vaccinia-F or -HN recombinant virus per cell and labeled with 100 μ Ci of [35 S]methionine per ml at 3 h p.i. For tunicamycin treatment, the drug (1 μ g/ml) was added 2.5 h before the addition of the radioactive label. Preparation of samples, immunoprecipitation, and polyacrylamide gel electrophoresis were done as described in Materials and Methods. Anti-F protein IgG and anti-HN IgG were described previously (14). Anti-vaccinia-F virus and anti-vaccinia-HN virus sera were prepared by infection of rabbits with purified recombinant viruses, as described in Materials and Methods. (A) Infected cells were either treated (+) or not treated (-) with tunicamycin. Lanes: 1 and 2, uninfected cells immunoprecipitated with anti-F IgG; 3 and 4, SV5-infected cells immunoprecipitated with anti-F IgG and anti-vaccinia-F virus sera, respectively; 5, vaccinia-F recombinant virus-infected cells immunoprecipitated with anti-F IgG; 6, SV5-infected cells treated with tunicamycin and immunoprecipitated with anti-vaccinia-F virus sera; 7, vaccinia-F recombinant virus-infected cells treated with tunicamycin and immunoprecipitated with anti-F IgG; 8 and 9, SV5-infected cells immunoprecipitated with anti-HN IgG and anti-vaccinia-HN virus sera, respectively; 10, vaccinia-HN recombinant virus-infected cells immunoprecipitated with anti-HN IgG; 11 and 12, SV5-infected cells without or with tunicamycin treatment, respectively, immunoprecipitated with anti-vaccinia-HN virus; 13 and 14, vaccinia-HN recombinant virus-infected cells without or with tunicamycin treatment, respectively, immunoprecipitated with anti-HN IgG. $\circ$, F₁ and F₂ at their appropriate positions; $\bullet$, unglycosylated F protein after tunicamycin treatment; $\rightarrow$, unglycosylated HN after tunicamycin treatment. (B) The following antisera were used: P, preimmune antisera; I, immune antisera; F, antisera from rabbits infected with vaccinia-F recombinant virus; HN, antisera from rabbits infected with vaccinia-HN recombinant virus; $\leftarrow$, F₀. SV5-infected CV1 cells were used as the antigen.

It can be seen in Fig. 1A, lane 3, that the anti-F-protein-specific IgG (13, 14) precipitated some HN from SV5-infected cells in addition to F₀, F₁, and F₂, as we had observed previously (25). The anti-HN IgG (13, 14) also precipitated some F₀, F₁, and F₂ from SV5-infected cells. Under identical immunoprecipitation conditions, the antibody raised to F protein expressed by vaccinia-F recombinant virus in rabbits precipitated only F-protein-related species (lane 4). Similarly, the antibody raised to HN expressed by vaccinia-HN recombinant virus in rabbits precipitated only HN (lane 9). Our data show that the nonspecificity of these early antisera cannot be attributed to the immunoprecipitation conditions but instead underscore the difficulty of purifying individual glycoproteins from paramyxoviruses.

Protective efficacy of vaccinia-F and vaccinia-HN viruses in an animal model. To determine whether antibodies to the individual F and HN proteins have a role in protection against SV5 infection, hamsters were inoculated intradermally with $10^{8.3}$ PFU of WT vaccinia, vaccinia-F, vaccinia-HN, or vaccinia-F plus vaccinia-HN viruses. In addition,

another group of animals received $10^{5.0}$ TCID₅₀s of SV5 intranasally. All of the 12 animals in each group survived the immunization scheme used with SV5, WT vaccinia virus, and the vaccinia virus recombinants. On day 28, blood was obtained from each hamster and assayed for SV5 antibodies by an SV5-specific ELISA, a hemagglutination inhibition assay, and a plaque reduction neutralization assay. The animals that received the vaccinia virus recombinants developed significant levels of antibody to SV5, as measured by ELISA, in comparison with the response induced by immunization with WT vaccinia virus (Table 1). However, immunization with vaccinia-HN virus was found to induce lower levels of antibody to SV5, as measured by ELISA, than did vaccinia-F or vaccinia-F plus vaccinia-HN viruses. Hamsters immunized with vaccinia-HN or vaccinia-F plus vaccinia-HN viruses possessed antibody that inhibited hemagglutination, although they had lower titers than did the animals that received SV5 intranasally. The groups of hamsters immunized with the vaccinia-F recombinant or WT vaccinia virus did not have detectable levels of antibody capable of inhibiting hemagglutination (Table 1). Represent-

TABLE 1. Immunogenicity and protective efficacy of vaccinia-F or vaccinia-HN recombinant virus in hamsters

Immunizing virus	Response to immunization ^a (reciprocal log ₂ antibody titer [mean $\pm$ SE]) on day 28 after immunization			Response to challenge ^b (log ₁₀ SV5 titer in lung [TCID ₅₀ /g] [mean $\pm$ SE])
	ELISA ^c	Hemagglutination inhibition	Neutralization	
Vaccinia WR	5.5 $\pm$ 0.2	$\leq 1.0 \pm 0.0$	$\leq 3.5 \pm 0.0$	5.3 $\pm$ 0.2
Vaccinia-F	13.3 $\pm$ 0.5	$\leq 1.0 \pm 0.0$	9.9 $\pm$ 0.5	4.0 $\pm$ 0.4
Vaccinia-HN	12.1 $\pm$ 0.3	4.3 $\pm$ 0.4	7.1 $\pm$ 0.5	3.0 $\pm$ 0.4
Vaccinia-F + vaccinia-HN	13.3 $\pm$ 0.2	3.8 $\pm$ 0.6	10.5 $\pm$ 0.5	2.3 $\pm$ 0.3
SV5	14.0 $\pm$ 0.3	7.9 $\pm$ 0.3	12.5 $\pm$ 0.2	$\leq 1.5 \pm 0.0$

^a Twelve hamsters per group were infected intradermally with $10^{8.3}$ PFU of WT vaccinia virus or recombinant virus(es) in a 0.1-ml inoculum. Twelve additional hamsters were intranasally infected with $10^{5.0}$ TCID₅₀s of SV5 in a 0.1-ml inoculum. Blood samples were collected before immunization and on day 28 after immunization.

^b On day 28, each hamster was challenged with $10^{5.0}$ TCID₅₀s of SV5 intranasally, and the lungs were harvested 4 days later (day 32) for determination of the level of virus replication.

^c There was a low level of background in the ELISA at the lowest dilution (1:40) tested. The level of reactivity was similar in pre- and postinfection sera.

tative serum samples from each group of animals were tested for the ability to inhibit cell fusion induced by SV5. Only serum samples from animals which received either SV5 or the vaccinia-F recombinant were found to have fusion inhibition activity (data not shown).

Serum from one animal from each test group was assayed for the ability to immunoprecipitate the SV5 F and HN polypeptides from [³⁵S]methionine-labeled SV5-infected-cell lysates. The data indicate that serum from the group immunized with vaccinia-F virus precipitated only F (Fig. 2, lanes 3), serum from the vaccinia-HN virus group precipitated HN (Fig. 2, lanes 5), and, most importantly, immunization with vaccinia-F plus vaccinia-HN viruses or SV5 induced antibodies that precipitated both F and HN (Fig. 2, lanes 4 and 6, respectively); the serum from the group immunized with WT vaccinia virus did not immunoprecipitate either of the SV5 surface antigens (Fig. 2, lanes 2).

All groups of hamsters except those that had been immunized

with WT vaccinia virus had serum-neutralizing antibody as measured by a 60% plaque reduction assay. Vaccinia-F virus, however, induced higher levels of neutralizing antibody than did vaccinia-HN virus (Table 1).

On day 28 after immunization, all groups of hamsters (12 in each group) were challenged with 10^5 TCID₅₀s of SV5 intranasally. Four days after the challenge, the animals were sacrificed, the lungs were removed, and the amount of virus in a 10% (wt/vol) lung suspension was determined (Table 1). It can be seen that immunization with the vaccinia virus recombinants induced resistance to SV5 challenge in comparison with immunization with WT vaccinia virus, although the hamsters that received the vaccinia-F and -HN recombinants were not protected quite as effectively as the group that had received SV5 intranasally.

DISCUSSION

The F and HN proteins expressed by the vaccinia recombinant viruses were indistinguishable in terms of electrophoretic mobility in both the presence and absence of tunicamycin (an inhibitor of N-linked glycosylation) from the F and HN proteins synthesized in cells infected with SV5. Immunization of rabbits with the vaccinia-F and vaccinia-HN recombinant viruses indicated that they were immunogenic and were able to induce circulating antibodies specific for the SV5 F or HN protein, as shown by immunoprecipitation of the proteins from SV5-infected cells. Each rabbit antiserum provides a useful reagent for further studies of the F and HN glycoproteins because it is truly a monospecific polyclonal antiserum.

Analysis of further properties of the antibodies induced by the vaccinia-F and -HN recombinant viruses was done using the Golden Syrian hamsters. The vaccinia virus recombinants were introduced intradermally either individually or together and compared with the controls, which consisted of groups of animals which received WT vaccinia virus intradermally or SV5 intranasally. Both the vaccinia-F and -HN recombinants induced antibodies as measured by ELISA, and the antibodies neutralized SV5, although the response to vaccinia-F virus was greater than that to vaccinia-HN virus in both instances. The level of SV5 ELISA antibody induced by vaccinia-F virus in conjunction with vaccinia-HN virus was equivalent to that induced by vaccinia-F virus alone, and this response was not significantly different from the SV5 ELISA antibody titer induced by SV5. However, the ELISA antibody titer induced by the vaccinia-HN recombinant was significantly less than that

FIG. 2. Immunoprecipitation analysis of antisera produced in Golden Syrian hamsters immunized with recombinant vaccinia viruses. Serum samples taken at day 0 or 28 after injection with vaccinia recombinant viruses were used to immunoprecipitate lysates of [³⁵S]methionine-labeled SV5-infected cells as described in Materials and Methods. Lanes: 1, markers showing HN and F precipitated with anti-vaccinia-HN virus and anti-vaccinia-F virus rabbit sera, respectively; 2, anti-WT vaccinia virus; 3, anti-vaccinia-F virus; 4, anti-vaccinia-F virus plus anti-vaccinia-HN virus from a hamster immunized with both vaccinia-F and vaccinia-HN viruses; 5, anti-vaccinia-HN virus; 6, anti-SV5.

resulting from intranasal inoculation of SV5. The results suggest that vaccinia-F virus is more immunogenic than vaccinia-HN virus, especially since the steady-state level of expression of F was three- to sevenfold lower than that of HN. As observed for ELISA antibody titers, vaccinia-F and vaccinia-HN viruses together induced levels of neutralizing antibodies similar to that induced by vaccinia-F virus alone.

Interestingly, although vaccinia-F virus induced higher levels of both neutralizing and ELISA antibodies than did vaccinia-HN virus, on challenge by SV5 it gave only equivalent or less protection. This result differs from those obtained in an analysis of the immune response to the G and F glycoproteins of the pneumovirus respiratory syncytial virus (RSV) (20). In the RSV study, cotton rats were immunized with vaccinia virus recombinants expressing the F and G proteins, and on subsequent challenge with RSV, the animals immunized with RSV-vaccinia-F virus were better protected than those that received RSV-vaccinia-G virus. The greater protection afforded by immunization with RSV-vaccinia-F virus was attributed to the higher titer of serum-neutralizing antibodies which RSV-vaccinia-F virus induced (20). The results presented here suggest that for SV5 there may be a qualitative difference in the *in vivo* properties of the neutralizing antibodies induced by the SV5-specific vaccinia-F and -HN viruses. In addition, the data indicate that there may be a significant contribution to immunity resulting from the stimulation of other humoral or cellular factors and that vaccinia-HN virus may be better at stimulating non-antibody-mediated immunity than is vaccinia-F virus; however, this has not been investigated.

In the past, studies on the immune response to paramyxovirus glycoproteins and the properties of the antibodies raised against them have given varied results concerning the extent of neutralization by anti-F serum (6, 10, 13, 18, 21–23, 31, 33). Most of these studies, using purified F protein as antigen, have found F antibody to have little or no neutralizing activity. In many instances, it is possible that F protein antibodies are nonfunctional (i.e., nonneutralizing) because of alterations to epitopes during purification of F (27). However, neutralizing anti-F protein antibody has been detected with canine distemper virus (18), mumps and Newcastle disease viruses (21), SV5 (13), and RSV (20). Our study with vaccinia virus-SV5 recombinants indicates that antibody induced by F expressed in host animals (i.e., "natural F") has considerable neutralizing activity. This might occur by blocking virus entry into cells or limiting the spread of virus by fusion from cell to cell or both.

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