

IMPORTANCE OF ANTIBODIES TO THE FUSION  
GLYCOPROTEIN OF PARAMYXOVIRUSES  
IN THE PREVENTION OF SPREAD OF INFECTION\*

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Paramyxoviruses comprise a large family of enveloped RNA viruses that include the parainfluenza, mumps, measles, and respiratory syncytial viruses (1). These viruses cause a variety of diseases, ranging in severity from mild upper respiratory infections to bronchiolitis, pneumonia, and encephalitis. They can also establish persistent infections (2, 3) that can lead to chronic disease, e.g., subacute sclerosing panencephalitis caused by measles virus (4). In spite of the importance of these diseases, effective live virus vaccines are available for only two of the human paramyxovirus infections, measles and mumps, and inactivated vaccines have been ineffective or have led to complications, as will be discussed below.

Two glycoproteins are found on the surface of paramyxoviruses (5) and are involved in the generation of immunity to infection. The HN<sup>1</sup> protein is responsible for hemagglutinating and neuraminidase activities and for adsorption of the virus to receptors on host cells (6). The F protein is involved in the cell-fusing and hemolyzing activities of the virus, and in virus penetration by fusion of viral and cell membranes (7-9). Activation of these functions results from a specific cleavage by a host protease of a precursor, F<sub>0</sub>, to yield the active F protein comprised of two disulfide-linked polypeptides (7-10). The cell-fusing activity of paramyxoviruses makes possible spread of infection from cell to adjacent cell by fusion of their plasma membranes, in addition to the more usual mode of dissemination of infection by released virus. The formation of multinucleated giant cells by cell fusion is a major cytopathic effect of paramyxoviruses (11, 12), and different cell types vary in their susceptibility to cell fusion (12-14). It is also pertinent to these studies that the virus-induced syncytia contain large amounts of viral proteins, even though little mature infectious virus may be released (12-14).

Because of the biological properties of the two paramyxovirus surface glycoproteins, antibody-mediated impairment of the function of either glycoprotein would be expected to affect the spread of a paramyxovirus infection. We have prepared

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<sup>1</sup> *Abbreviations and nomenclature used in this paper:* BS, buffered saline; F, viral glycoprotein with membrane-fusing activity; FITC-GARiG, fluorescein isothiocyanate-conjugated goat (anti-rabbit IgG) IgG; H, hemagglutinin protein; HN, viral glycoprotein with hemagglutinating and neuraminidase activities; MDBK, Madin-Darby bovine kidney; MEM, Eagle's minimal essential medium; MOI, multiplicity of inoculation; PFU, plaque-forming units; p.i., postinfection; PSA, phosphate-buffered saline azide; REM, reinforced Eagle's minimal essential medium; RS, respiratory syncytial; SV5, simian virus 5.

antibodies specific for each of the glycoproteins of the parainfluenza virus simian virus 5 (SV5)<sup>2</sup> to study the functions of these proteins and to evaluate the effectiveness of antibodies to each in inhibiting the individual biological activities of the proteins and in preventing the initiation and spread of infection. This report describes the effects of these antibodies on the spread of SV5 infection in two cell types that differ in their susceptibility to virus-induced cell fusion. It will be shown that anti-HN antibodies, which block virus adsorption, were effective only when there was little cell fusion and dissemination occurred through released progeny virions, whereas anti-F antibodies were capable of completely preventing the spread of infection in cells susceptible to virus-induced fusion, as well as in nonfusing cells. These results, which emphasize the importance of cell-fusing activity in the spread of paramyxovirus infections, are discussed in relation to the requirements for effective vaccines against paramyxovirus infections, the failure of inactivated vaccines, and the serious atypical diseases that may occur when individuals immunized with inactivated vaccines are infected with live virus.

### Materials and Methods

*Cells and Virus.* The Madin-Darby bovine kidney (MDBK) cells were grown in reinforced Eagle's minimal essential medium (REM) with 10% fetal bovine serum (6). An epithelioid clone of the CV-1 line of African green monkey cells, kindly provided by Dr. E. Gershey (The Rockefeller University, New York), was grown as described<sup>3</sup> in Eagle's minimal essential medium (MEM) with 10% fetal bovine serum. The growth of stocks of SV5 in MDBK cells and the determination of infectivity by plaque assay in MDBK or CV-1 cells have been described previously (6, 9).<sup>2</sup>

*Antibodies.* The preparation and characterization of antiviral glycoprotein antibodies have been described in detail elsewhere.<sup>2</sup> Briefly, rabbits were immunized with nonionic detergent-disrupted SV5 virions in complete Freund's adjuvant, and high-titer antisera ( $\alpha$ SV5) against all the viral structural proteins were obtained. The virus used as immunogen was grown in the HKCC line of hamster kidney cells to avoid the possibility of cross-reaction with cellular antigens in experiments in MDBK or CV-1 cells.  $\alpha$ HN and  $\alpha$ F antisera specific for the individual glycoproteins were prepared similarly, using as immunogens purified glycoproteins obtained by adsorption chromatography on fetuin-agarose of nonionic detergent-solubilized viral envelope proteins.<sup>2</sup> The specificities of the antibodies were shown by double immunodiffusion, immunoprecipitation, and the appropriate specific inhibition of viral hemagglutinating, neuraminidase, hemolyzing, and cell-fusing activities.<sup>2</sup> All experiments were done with isolated IgG or Fab fragments as indicated in the text. IgG was isolated from all sera by ion-exchange chromatography (15) on DEAE-cellulose (DE-52; Whatman, Inc., Clifton, N. J.), and stored at 4°C in phosphate-buffered saline azide (PSA; 0.05 M sodium phosphate, pH 7.35, 0.15 M NaCl, 0.02% [wt/vol] sodium azide). Fab fragments of  $\alpha$ HN and  $\alpha$ F antibodies were prepared by digestion with papain (16) (Worthington Biochemicals Corp., Freehold, N. J.). Before use, antibodies were dialyzed against two changes of 1,000 vol of MEM or REM at 4°C, and then sterilized by ultrafiltration through a Millipore filter (type HA; Millipore Corp., Bedford, Mass.).

*Neutralization of the Spread of Infection.* Confluent monolayers of CV-1 or MDBK cells in microtest plates (No. 3034; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) were inoculated with SV5 at a multiplicity of inoculation (MOI) of 0.01 plaque-forming units (PFU) per cell. After a 2-h adsorption period, cells were washed with MEM or REM, and 10  $\mu$ l of medium was placed in each well. The medium in duplicate plates was replaced 9 h

<sup>2</sup> Merz, D. C., A. Scheid, and P. W. Choppin. 1979. Immunochemical studies on the functions of the glycoproteins of the paramyxovirus SV5. Manuscript submitted for publication.

<sup>3</sup> D'Alisa, R. M., and E. L. Gershey. 1979. Characterization of a CV-1 cell cycle. I. Definition of a synchrony system. Manuscript submitted for publication.

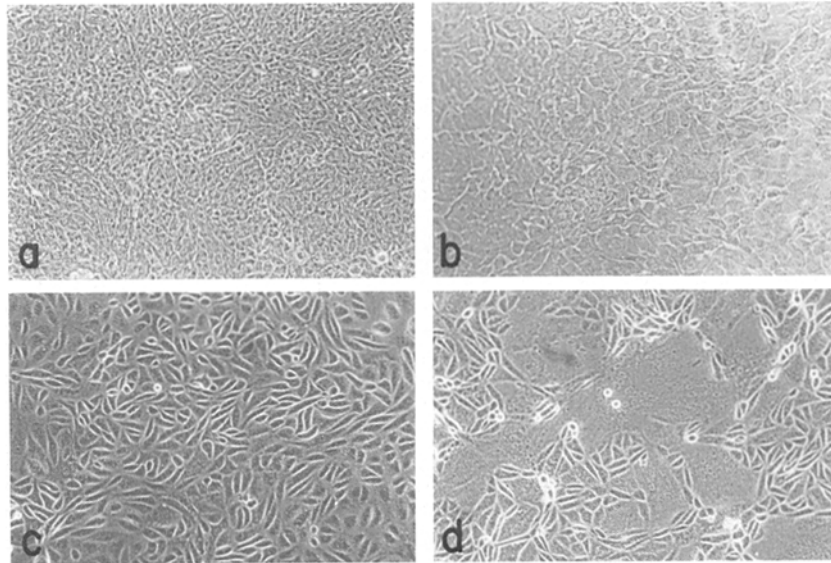


FIG. 1. Cytopathic effects in SV5-infected MDBK (a and b) or CV-1 (c and d) cells. (a) and (c), mock-infected cells. (b) MDBK cells 36 h p.i. (d) CV-1 cells 24 h p.i. Cells were infected at an MOI of 1 PFU/cell. Phase-contrast micrograph.  $\times 67$ .

postinfection (p.i.) with medium that contained either preimmune rabbit IgG, or anti-glycoprotein IgG or Fab, and incubation was continued at 37°C. Anti-glycoprotein antibodies were used in twofold dilutions made from 2 mg/ml stock solutions in MEM or REM. At 30–32 h, and again at 50–60 h p.i., the monolayers in one plate were washed repeatedly with buffered saline (BS; 0.02 M sodium phosphate, pH 7.2, 0.15 M NaCl), and fixed with chilled (–20°C) ethanol for 15 min at –70°C. The plates were air dried and stored at –70°C until immunofluorescent staining was performed.

*Indirect Immunofluorescent Staining.* Fixed cell monolayers were rehydrated for 30 min at 25°C with BS, and after aspiration, each well received 5  $\mu$ l of preimmune rabbit IgG or  $\alpha$ SV5 IgG, at a concentration of 2 mg/ml in PSA. Plates were incubated for 1 h at 37°C in a humidified chamber. After washing excess unbound IgG from the plates with BS and aspiration of BS to near dryness, 5  $\mu$ l of fluorescein isothiocyanate-conjugated goat (anti-rabbit IgG) IgG (FITC-GAR IgG; fluorescein:protein molar ratio = 4.34; Antibodies, Inc., Davis, Calif.) at a concentration of 0.5 mg/ml in PSA, was added to each well, and incubation was continued at 37°C for an additional hour. Unbound FITC-GAR IgG was washed from each well with BS. Stained plates were stored in BS at 4°C in the dark until they were examined and photographed with a Leitz inverted microscope (E. Leitz, Inc., Rockleigh, N. J.) with epifluorescent excitation at 495 nm and suppression at 525 nm. Kodak Tri-X (Eastman Kodak, Co., Rochester, N. Y.) was used at ASA 1,600 and developed with Diafine (Acufine, Inc., Chicago, Ill.). Exposures were automatically metered, and photomicrographs were taken at a magnification of 40.

## Results

*Progression of SV5 Infection in MDBK and CV-1 Cells.* Paramyxovirus infection may be propagated by the conventional mode in which virions released from an infected cell are disseminated in the extracellular environment to adsorb to and penetrate other cells. In addition, because of their ability to cause cell fusion, paramyxoviruses can spread directly to adjacent cells by fusion of their membranes, forming syncytia that can become very large. Fig. 1 shows cytological changes in two cell types in which the two different modes of spread of infection occur. MDBK cells yield large

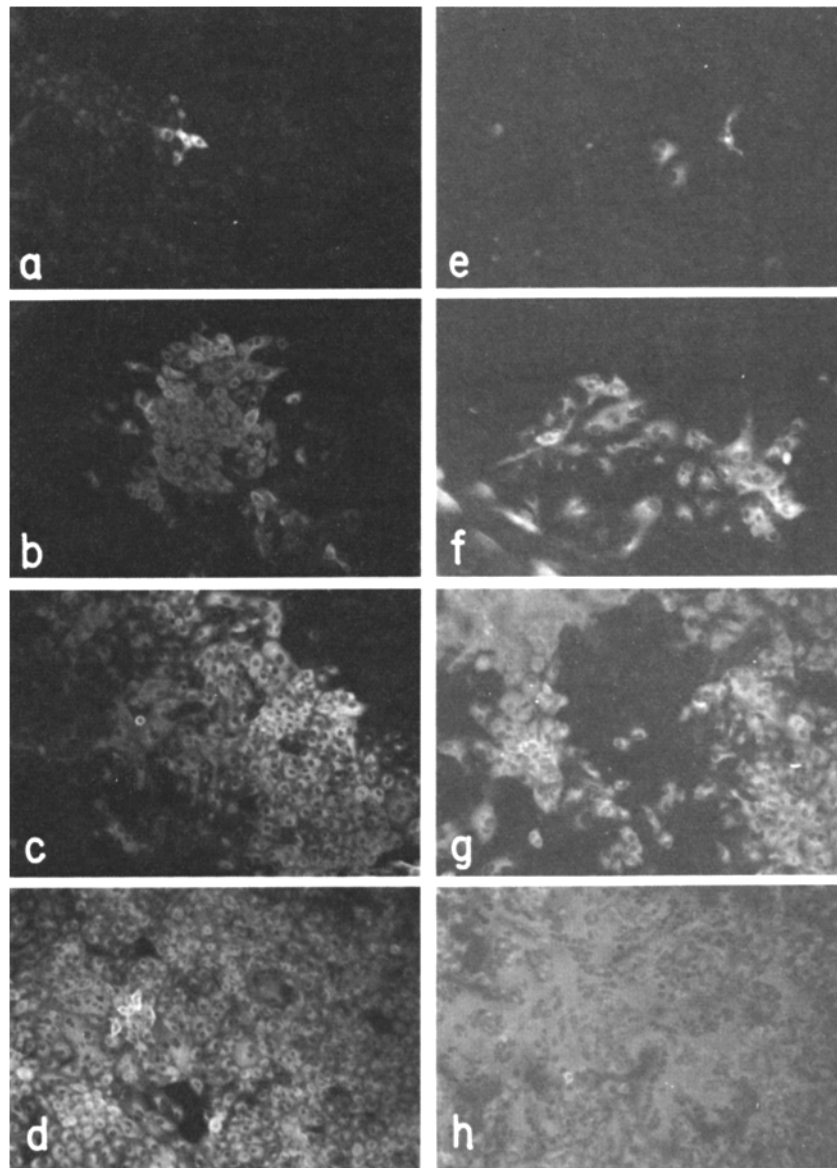


FIG. 2. The spread of SV5 infection in MDBK and CV-1 cells shown by immunofluorescence using antiviral antibodies. Left: MDBK cells (a) 30 h, (b) 40 h, (c) 50 h, and (d) 65 h p.i. Right: CV-1 cells (e) 24 h, (f) 42 h, (g) 56 h, and (h) 67 h p.i. MOI, 0.01 PFU/cell. Indirect immunofluorescent staining with rabbit  $\alpha$ SV5 IgG and FITC-GAR IgG.  $\times 67$ .

amounts of virus, e.g.,  $\sim 600$  PFU/cell in 24–36 h with production continuing for several days (6, 14), and they are relatively resistant to virus-induced cell fusion. Fig. 1 b shows MDBK cells 36 h after infection; they appear somewhat larger than control cells (Fig. 1 a), but there are no giant cells and the monolayer is still intact. In contrast, SV5-infected CV-1 cells produce  $\sim 100$  PFU/cell and undergo extensive cell fusion, leading to the formation of large syncytia (Fig. 1 d) and subsequent cell death. Similar

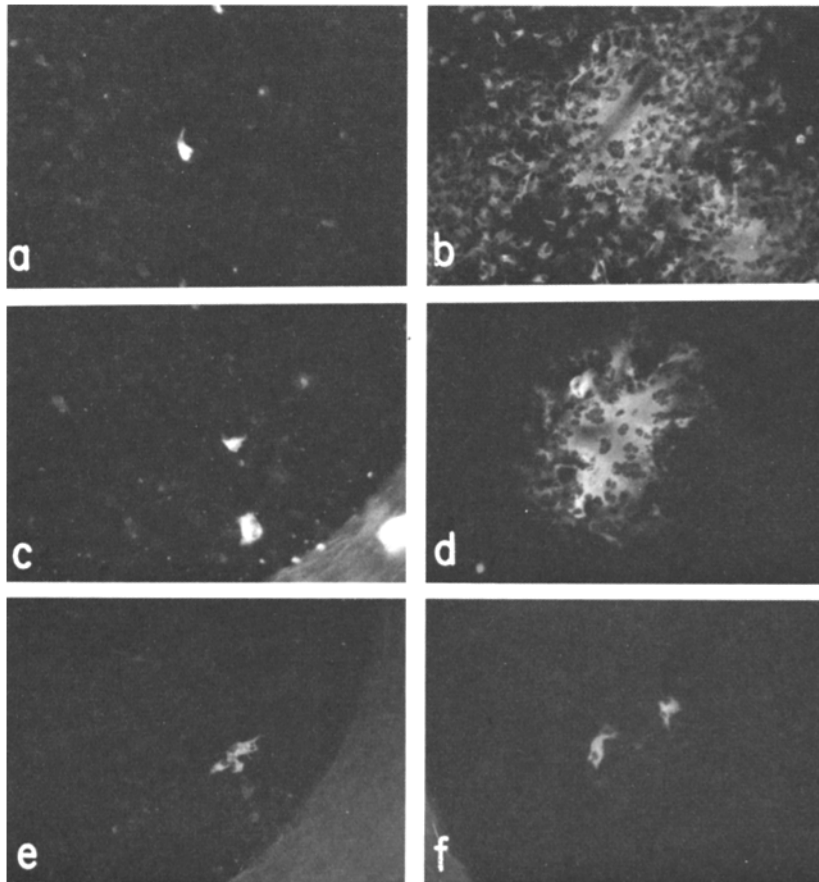


FIG. 3. Neutralization of the spread of SV5 infection in CV-1 cells with preimmune (a, b),  $\alpha$ HN (c, d), or  $\alpha$ F (e, f) IgG that were added 9 h p.i. Cells were stained with rabbit  $\alpha$ SV5 and FITC-GAR IgG at 30 h (a, c, e) and 50 h (b, d, f) after infection at an MOI of 0.01 PFU/cell. Concentrations of antibodies used for neutralization were: preimmune 2 mg/ml;  $\alpha$ HN and  $\alpha$ F, 1 mg/ml.  $\times 67$ .

differences in the susceptibility of different cells to SV5-induced cell fusion, and the correlation of such differences with cell survival and virus production, have been described previously (3, 12-14).

The time-course of SV5 infection in MDBK and CV-1 cells, as detected by immunofluorescent staining with  $\alpha$ SV5 antibody, is illustrated in Fig. 2. Cells were inoculated with a low virus multiplicity to give multiple-cycle replication and to permit the observation of the development of foci of infection. In MDBK cells (Fig. 2 a-d), single, brightly stained cells were detected 30 h p.i., and in the ensuing 35 h the infection was disseminated to the entire cell monolayer. The vast majority of infected cells remained as single cells, and, although there were a very few binucleate cells, there was no evidence of extensive cell fusion. There was no staining of the nuclei of infected cells, and this is in agreement with the previous demonstration that the replication of SV5 is confined to the cytoplasm (12, 13).

In infected CV-1 cells (Fig. 2 e-h), single stained cells were detected at 24 h, and by 42 h there were scattered single infected cells, whereas most of the infected foci were

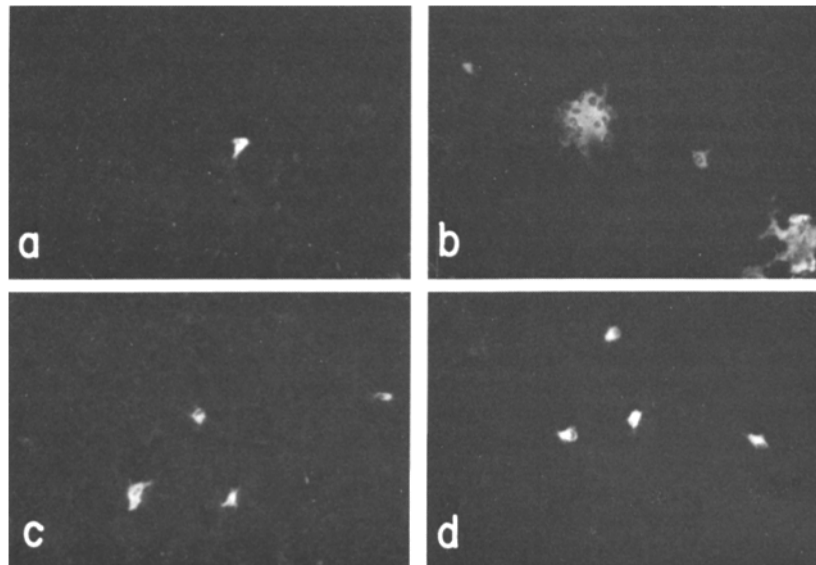


FIG. 4. Neutralization of the spread of SV5 infection in CV-1 cells by monovalent antibodies added 9 h p.i. (a, b)  $\alpha$ HN Fab; (c, d)  $\alpha$ F Fab. Cells were stained with  $\alpha$ SV5 IgG and FITC-GAR IgG at 32 h (a, c) and 58 h (b, d) after infection at an MOI of 0.01 PFU/cell.  $\alpha$ HN Fab and  $\alpha$ F Fab concentrations, 1 mg/ml.  $\times 67$ .

multinucleate giant cells (Fig. 2 e), which enlarged to involve the entire monolayer by 67 h (Fig. 2 h). These studies illustrate that whereas in MDBK cells the infection is disseminated from cell to cell by released virus without cell fusion, in CV-1 cells a major mode of spread is by fusion of adjacent cells resulting in the formation of enlarging syncytia. Dissemination of infection to noncontiguous cells by released virus can also occur in CV-1 cells.

*Neutralization of the Spread of SV5 Infection in Fusing Cells with Monospecific Antibodies against the Individual Virus Glycoproteins.* The ability of antibodies against the HN or F glycoproteins of SV5 added after the viral adsorption period to prevent the spread of infection in CV-1 cells was investigated. It was previously established<sup>2</sup> that either of these antibodies can neutralize the infectivity of the virus if added to the virus before adsorption; the  $\alpha$ HN antibodies inhibit adsorption, and the  $\alpha$ F antibodies inhibit virus penetration that is mediated by virus-cell membrane fusion. Fig. 3 a and b show the normal progression of infection in the presence of preimmune IgG, with large syncytia as well as single infected cells present. Fig. 3 c and d show that  $\alpha$ HN antibodies, although inhibiting the dissemination of infection to single cells, do not prevent the spread of infection by cell fusion and the formation of large syncytia by 50 h p.i. In contrast, the  $\alpha$ F antibody completely prevented the spread of infection, i.e., the infection was confined to the few individual cells that were initially infected, and there were no syncytia.

These results are consistent with the known activities of the HN protein. By preventing virus adsorption, which is mediated by the HN protein,  $\alpha$ HN antibodies effectively prevented the dissemination of infection by released virus, but did not inhibit spread by cell fusion, which is mediated by the F protein. On the other hand,  $\alpha$ F antibodies were capable of completely preventing the spread of infection by

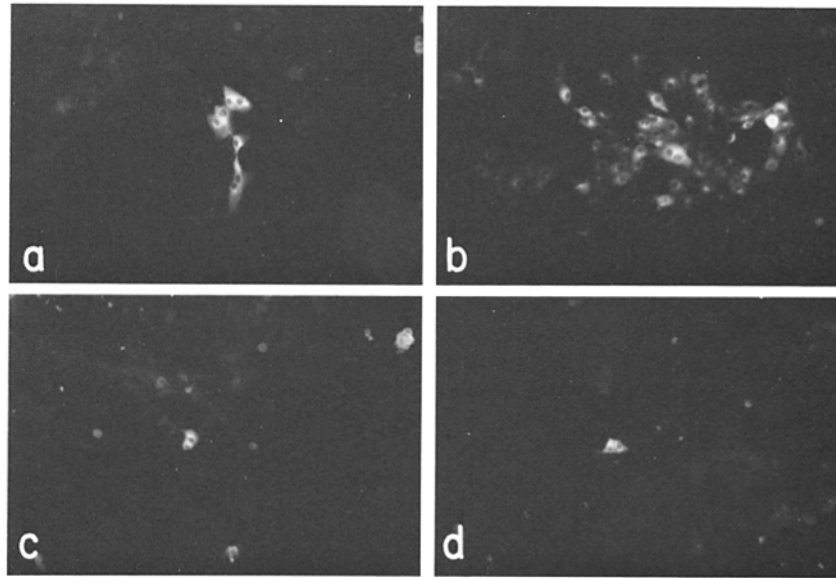


FIG. 5. Neutralization of the spread of SV5 infection in MDBK cells by monovalent antibodies added 9 h p.i. (a, b) preimmune IgG; (c, d)  $\alpha$ HN Fab. Cells were stained with  $\alpha$ SV5 IgG and FITC-GAR IgG at 36 h (a, c) and 58 h (b, d) after infection at an MOI of 0.01 PFU/cell. Concentration of antibodies: preimmune IgG, 2 mg/ml;  $\alpha$ HN Fab, 1 mg/ml.  $\times 67$ .

blocking the cell-fusing activity of the F protein, as well as neutralizing any released virus at the level of virus penetration, a step in infection which results from fusion of viral and cell membranes mediated by the F protein. The failure of the  $\alpha$ HN antibodies to inhibit syncytium formation was not simply a result of the lack of sufficient antibody to neutralize the released virus, because the  $\alpha$ HN IgG preparation used contained twice the neutralizing capacity of the  $\alpha$ F IgG in a preadsorption neutralization test.<sup>2</sup> Furthermore, as shown below, the  $\alpha$ HN serum was capable of preventing spread of infection in cells that were resistant to fusion. Thus the spread of infection in the presence of  $\alpha$ HN antibodies was possible because of the uninhibited action of F protein, which was capable of causing fusion of infected cells with adjacent cells, allowing spread without the release of virus into the medium.

It has been reported that under certain conditions virus-specific antisera may enhance the extent of cell fusion in paramyxovirus-infected cells (17, 18). Thus, the cell fusion seen in the presence of  $\alpha$ HN IgG might not have been only the result of unimpaired expression of F glycoprotein activity, but also the result of immune complex formation and consequently improved cell-fusing activity. To investigate this possibility, similar neutralization of dissemination experiments were repeated with monovalent  $\alpha$ HN and  $\alpha$ F antibodies. It was shown previously that Fab fragments possessed the same activities against virions as did bivalent IgG antibodies.<sup>2</sup> As shown in Fig. 4, the presence of  $\alpha$ HN or  $\alpha$ F Fab in the medium after inoculation gave results that were identical to those obtained above with IgG.  $\alpha$ HN Fab (Fig. 4 a and b) prevented dissemination by released virus but did not inhibit the spread of infection by cell fusion, whereas  $\alpha$ F Fab (Fig. 4 c and d) prevented entirely the spread of infection. Hence, the spread of infection by fusion of adjacent cells in the presence of  $\alpha$ HN antibody was not the result of aggregated progeny virions with increased fusion

capability, but simply fusion of cells through the unrestricted action of F glycoprotein.

*Neutralization of Virus Dissemination in Nonfusing Cells with Monospecific Antibodies against Viral Glycoproteins.* Cell-to-cell spread of infection in nonfusing cells in the absence of released infectious virions would be expected to be minimal. To investigate this question, the effect of antibodies against the individual viral glycoproteins on the dissemination of infection in MDBK cells was determined. As shown above (Fig. 1 b), extensive fusion does not occur in SV5-infected MDBK cells. Control preimmune rabbit IgG failed to alter the progression of the infection (Fig. 5 a and b); however, the presence of either  $\alpha$ HN Fab (Fig. 5 c and d) or  $\alpha$ HN IgG (not shown) in the medium after infection completely prevented the spread of infection. As expected from the above results with CV-1 cells, both  $\alpha$ F IgG and  $\alpha$ F Fab also completely prevented the spread of infection in MDBK cells (not shown). Thus, in the absence of cell fusion, HN antibodies are capable of inhibiting the dissemination of the virus, and it is the ability of the F protein to cause cell fusion that allows the virus to spread in the presence of antibodies to the HN glycoproteins.

#### Discussion

The use of monospecific antibodies against each of the two surface glycoproteins of a paramyxovirus has enabled a better understanding of the role of these proteins in the initiation and propagation of infection and has demonstrated the requirement for antibodies to the F glycoprotein for the complete prevention of spread of paramyxovirus infections. Previous studies have shown that the HN protein is required for virus adsorption, and that the F protein is responsible for the virus-induced fusion of membranes that is essential for virus penetration, virus-induced hemolysis, and the formation of syncytia in infected cells (7-9, 19). In addition, it has been shown that antibodies against either of the glycoproteins are capable of neutralizing the infectivity of the virus if the antibody interacts with the virus before it adsorbs to the cell, the  $\alpha$ HN antibodies by preventing virus adsorption, and the  $\alpha$ F antibodies by inhibiting penetration.<sup>2</sup> The studies reported here have illustrated that a paramyxovirus can spread from cell to cell by means of cell fusion without a requirement for released infectious virus. The use of monospecific antibodies has shown that such spread is the result of the action of the F protein and that antibodies to this protein are required to prevent spread of infection in a system in which cell fusion can occur, whereas antibodies to the HN protein can prevent dissemination of infection through neutralization of released virus only if cell fusion does not occur.

The requirement for antibodies to the F protein for the prevention of spread of paramyxovirus infections has significant implications for the development of effective paramyxovirus vaccines, and may provide an explanation for previous failures of inactivated vaccines and the complications encountered when certain individuals who received such vaccines were infected with the respective virus. Formalin-inactivated vaccines and, in a few cases, Tween-ether-inactivated vaccines, have been prepared and tested for measles, mumps, parainfluenza, and respiratory syncytial (RS) viruses (20-36). These vaccines have proven not to be successful, even in cases in which they induced the formation of significant titers of hemagglutination-inhibiting or neutralizing antibodies, as determined in conventional tests in which virus and sera are mixed before virus adsorption. Not only was there no effective protection with these vaccines, but in some instances, e.g., with measles and RS viruses, the illness that



developed on subsequent infection was more severe than in unvaccinated individuals (22, 23, 25–28, 32–36). This will be discussed further below.

Evidence that suggested an explanation for the failure of inactivated vaccines was obtained by Norrby and co-workers (29–31, 37), who found that formalin- or Tween-ether-inactivated measles and mumps vaccines induced hemagglutinating-inhibiting, but not hemolysis-inhibiting antibodies in humans, and that inactivated Sendai virus, a murine parainfluenza virus, produced similar results in rabbits. These workers suggested that the failure of the vaccines could be related to the lack of hemolysis-inhibiting antibodies, and that this lack might also be somehow involved in the atypical measles that occurred in individuals receiving inactivated vaccine (30). In interpreting these results with measles virus, it is pertinent that recent studies with this virus have indicated that the protein responsible for measles virus-induced hemolysis is analogous to the F protein of other paramyxoviruses, and that the hemagglutinin protein (H) of measles is analogous to the HN of other paramyxoviruses except that it does not possess neuraminidase activity (38, 39).

Our finding of the requirement for antibodies to the F protein for the complete prevention of the spread of a paramyxovirus infection, coupled with the failure of formalin-inactivated vaccine to stimulate the formation of antibodies to the F protein, provides not only an explanation for the failure of such vaccines to provide effective protection, but also additional information on the pathogenesis of pneumonia in atypical measles and the severe bronchiolitis and pneumonia that have been observed in some patients who were immunized with formalin-inactivated vaccines and subsequently infected. In both instances it has been suggested that these syndromes involved immunopathological processes. Buser (32) and Scott and Bonnano (33) observed local reactions in individuals who received killed-measles virus vaccine and later live measles virus vaccine that were suggestive of Arthus reactions, and subsequent studies have supported this conclusion (34). Chanock et al. (35) found that the highest evidence of RS virus pneumonia occurred in infants with the highest level of neutralizing antibodies in their serum, consistent with an antibody-mediated pathogenesis of the disease, and suggested that, in the bronchiolitis occurring in children who had received the killed RS virus vaccine, the severity of the disease was a result of reaction between the circulating antibodies elicited by the vaccine and virus replicating in the lung because of the absence of local immunity (36). Additional evidence suggesting that immunopathological mechanisms may play a role in paramyxovirus infections has been obtained in studies of respiratory infections of children (40), cattle (41), and mice (42), which implicated virus-specific immune complex formation in pathogenesis, and in the finding that experimentally induced immune complex disease of the lung resembles viral interstitial pneumonia (43, 44).

Correlation of our demonstration that  $\alpha$ F antibodies are required to completely prevent the spread of paramyxovirus infections with the previously observed lack of  $\alpha$ F antibodies in individuals receiving inactivated vaccines and the clinical findings mentioned above has led us to suggest a hypothetical explanation for the atypical disease occurring in recipients of killed-virus vaccines that is schematically depicted in Fig. 6. Some cells in the respiratory tract are infected in the initial exposure to the virus, and there could perhaps be some early dissemination by released virus as a result of the absence of local immunity. The infection could undergo spread from the initially infected cells to adjacent cells by cell fusion because antibody to the receptor-

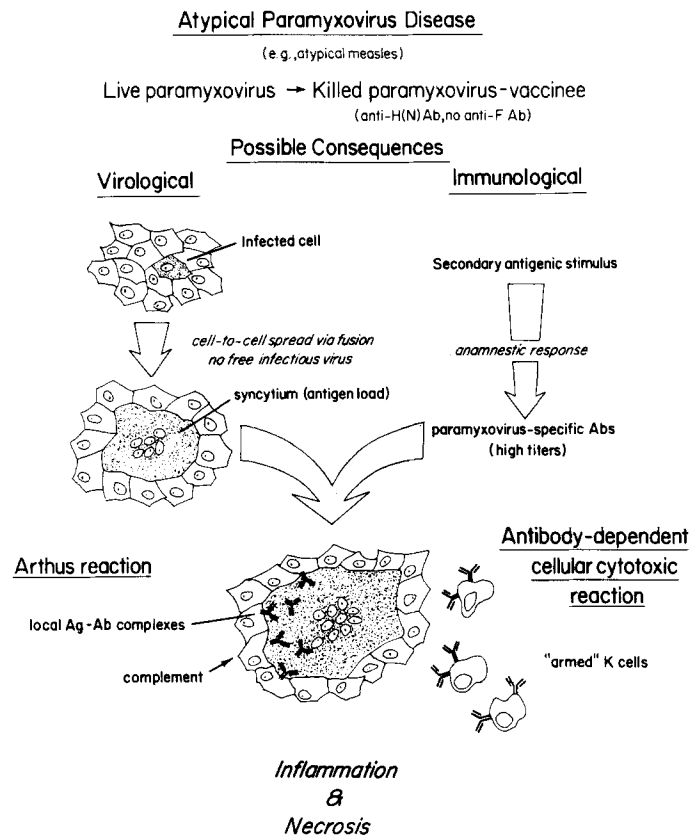


FIG. 6. Schematic representation of a hypothetical sequence of events leading to immunopathological injury in individuals infected with a paramyxovirus after vaccination with an inactivated virus vaccine that failed to stimulate antibodies to the viral F glycoprotein.

binding protein (HN, or H in the case of measles) present as a result of vaccination could not prevent the spread by fusion. It is known that paramyxoviruses can cause syncytium formation in respiratory epithelium. This is maximally expressed in the giant cell pneumonia that occurs in immunocompromised hosts infected with measles (45, 46) or parainfluenza 3 (47, 48) viruses. As the infection spreads by cell fusion, the viral antigens produced in those cells, and released virus particles neutralized by the  $\alpha$ H antibodies, would serve as secondary antigenic stimuli, resulting in a hyperimmune response to H and other viral antigens (except F) for which the vaccination had provided the primary stimulus. Recent studies have shown that the convalescent sera of patients with atypical measles contain high levels of antibodies, not only to the H protein, but also to other viral proteins (49) (W. W. Hall, M. H. Kaplan, P. W. Choppin. Unpublished observations.). With the infected syncytia presenting the viral antigens, immune complex formation could occur in the lungs, resulting in complement activation and inflammation, i.e., an Arthus-like reaction. Alternatively, K cells could be recruited to antibody-coated syncytia (50–53), resulting in an antibody-dependent cellular cytotoxic reaction. This sequence of events could explain atypical measles in killed-virus vaccinees. A similar situation could be obtained with RS virus.

It is not yet clear which RS viral protein is responsible for cell fusion, but there can be little doubt that a viral protein is involved, and it would appear likely from the experience with measles, mumps, and parainfluenza viruses that formalin treatment could inactivate the antigenicity of the putative RS virus fusion protein as well.

The studies reported here have clearly demonstrated that an effective vaccine against paramyxoviruses must elicit antibodies to the F protein. Live virus vaccines would be expected to do so, and much effort is being devoted to the development of live virus vaccines for several of these viruses for which vaccines are not now available, including RS virus (54). However, there are factors that argue for development of inactivated vaccines as well, e.g., the availability of a vaccine for use in immunocompromised individuals, and the theoretical possibility of persistent infection after vaccination, a problem that deserves particular consideration with paramyxoviruses because of the ability of many of them to cause persistent infections (1, 2). The failure of previous formalin-treated vaccines against several paramyxoviruses has understandably dampened enthusiasm for inactivated paramyxovirus-vaccines; however, because these vaccines were deficient in eliciting  $\alpha$ F antibodies, and it is now clear that  $\alpha$ F antibodies are essential for effective protection, this situation should be reevaluated. Vaccines consisting of isolated purified viral surface proteins, not requiring formalin treatment, could represent important additions to our immunization armamentarium against these important diseases.

#### Summary

The effects of monospecific antibodies to the viral glycoprotein with hemagglutinating and neuraminidase activity (HN) and the viral glycoprotein with membrane-fusing activity (F) of the paramyxovirus simian virus 5 (SV5) on the spread of infection in two cell types have been investigated. In CV-1 cells, infection can spread by either released progeny virus adsorbing to and infecting other cells, or by fusion of an infected cell with an adjacent cell as a result of the cell-fusing activity of the F glycoprotein. In these cells, antibodies specific for the HN glycoprotein prevented the dissemination of infection by released infectious virus, but spread by cell fusion was not inhibited. Antibodies to the F glycoprotein completely prevented the spread of infection in these cells. In Madin-Darby bovine kidney cells, which are relatively resistant to SV5-induced fusion, antibodies to either the HN or F glycoproteins were capable of preventing the dissemination of infection.

These results indicate that effective immunological prevention of the spread of paramyxovirus infection requires the presence of antibodies that inactivate the F glycoprotein. This requirement for anti-F antibodies has obvious implications for the design of effective paramyxovirus vaccines and provides an explanation for previous failures of formalin-inactivated paramyxovirus vaccines as well as additional insight into the possible immunopathological mechanisms involved in the atypical and severe infections that have occurred in individuals who received inactivated paramyxovirus vaccines and were subsequently infected by the virus.

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