Infection-Mediated Resistance to Cell Fusion by Inactive Sendai Virus[1] (37158)

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Infection of susceptible cells with myxoviruses and paramyxoviruses is dependent on the presence at the cell surface of specific sialoglycoprotein receptors. Insofar as it has been determined, the same receptor complex serves for all myxoviruses and paramyxoviruses and is similar to erythrocyte receptors through which hemagglutination and, with paramyxoviruses, hemolysis and cell fusion, are effected. Accordingly, Okada (1) demonstrated that *Vibrio cholerae* neuraminidase (receptor destroying enzyme, RDE) rendered Ehrlich ascites tumor cells insusceptible to fusion by inactive Sendai virus. In addition, the same author demonstrated that energy reserves in the form of nucleoside triphosphate, also present in the membrane, are required for cell fusion (1). Other than the foregoing, relatively little is known about conditions or factors at the surface of cells which may influence their susceptibility to extrinsic viral fusion. We have attempted to determine what effect the active synthesis of each of several viruses may have on the susceptibility of the infected cell membrane to the fusing action of Sendai virus. Certain agents were chosen because of involvement of membranes in their own maturation and/or release (myxoviruses, pseudomyxoviruses, Sindbis, vesicular stomatitis and herpes simplex viruses). Others, exhibiting less well-defined or no relationship to the plasma membraned, served by way of controls (vaccinia virus, poliovirus and adenovirus).

Materials and Methods. Type 2 parainfluenza virus (Pfl 2, clone 13) was grown in FL cells (2). Respiratory syncytial virus (RS virus) was maintained by serial passage in VERO, FL, or HEp-2 cells. Measles virus (Edmonston strain) was grown for several generations in VERO cells before passage in FL cells. Herpes simplex virus (HSV), adenovirus type 7 (AV7), poliovirus (type 1, Sabin) vesicular stomatitis virus (VSV), vaccinia virus and Sindbis virus were grown in FL cells. For preparation of stock virus, confluent monolayers were infected with passage material at multiplicities less than 0.1. Cultures were maintained in BME, enriched with 0.1% lactalbumin hydrolysate and 2% calf serum. Cell-associated virus was harvested by three cycles of freezing and thawing of cells scraped from the vessel surface and mixed with supernatant fluid. Cell debris was removed by centrifugation for 60 min at 1500*g*. Sendai virus and the PR8 strain of influenza A virus were maintained by serial passage in eggs.

For plaque assays, adsorption to cell monolayers in plastic flasks (Falcon) was carried out for 2 hr at 37° with each virus except RS virus, for which a 3-hr adsorption was employed. Excess inoculum was then removed, and each cell sheet overlaid with appropriate medium. For VSV, HSV, poliovirus, vaccinia and measles viruses, maintenance medium in 0.9% agar was employed. One to 3 days later, a second nutrient overlay containing 0.1% neutral red was added to the monolayers and plaques were counted after a further 24 hr incubation. Pfl 2 virus was assayed by means of hemadsorption plaque counts as previously described (2). RS virus, AV7 and Sindbis virus stocks were titrated for CPE end points in replicate tube

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cultures of FL cells; 50% end points were calculated by the method of Reed and Muench (3).

Egg-grown Sendai virus concentrates were prepared as previously described (4) and were inactivated with 0.01% betapropiolactone according to the method of Neff and Enders (5). Complete inactivation of infectivity was verified by absence of hemadsorption in chicken embryo fibroblast cultures inoculated with BPL-treated viral concentrates, which retained full hemagglutinin and neuraminidase activities (4).

All viral antisera were prepared in rabbits, with the exception of antibody to measles which was obtained from Lederle Laboratories (reference serum No. 2175-474). Serum globulin fractions were conjugated with fluorescein isothiocyanate by Dr. K. C. Hsu, Columbia University.

Fusion assays. Replicate monolayers of FL or HEp-2 cells, grown to confluence on coverslips, were exposed to infecting virus for 2 hr at 37° on a mechanical rocker, and then challenged at various times with 0.2 ml BPL-inactivated Sendai virus. The challenge virus was used at the highest dilution which gave complete fusion of uninfected monolayers maintained under identical conditions. Two hours after challenge with Sendai virus, cells were fixed in methanol and stained with Lillie's azure-eosin. Fusion indices were expressed as the number of nuclei counted/the number of cells in which they were found. A minimum of 500 nuclei in 4 different fields were examined on each stained coverslip. Monolayers were also fixed in acetone at various times and stained with fluorescein-conjugated antisera to determine the extent of the primary viral infection and to follow the fate of viral antigen during the course of subsequent challenge with inactive Sendai virus.

Results. Cells preinfected with poliovirus 1, AV7, HSV, VSV, vaccinia or Sindbis viruses remained fusible throughout the entire cycle of replication of each virus (Fig. 1, a–f). Despite rapidly evolving cytopathic effects which resulted in the detachment of a proportion of cells from the vessel surface, those cells which remained attached were still fusible even in the late stages of the primary viral infection. However, the massive syncytia which resulted under these conditions were not viable, and disintegrated soon after medium was replaced. Control, uninfected syncytia, on the other hand, could be maintained for several weeks, without apparent loss of viability. Both HSV and vaccinia virus were relatively nonsyncytiogenic in FL cells. Therefore, any fusion observed after challenge of monolayers infected by either of these two agents could be attributed to the action of the challenge Sendai virus.

In contrast to the foregoing, prior infection of FL cells with influenza or parainfluenza viruses (either type 1 or type 2) caused in each case a progressive diminution in susceptibility to extrinsic fusion by Sendai virus (Fig. 2, a–c). Resistance in each case appeared concomitantly with newly synthesized viral hemagglutinin, hemadsorption and specific fluorescence; the presence of newly synthesized neuraminidase in the membrane as well as on released progeny virus could be assumed under these circumstances. Viral antibody added to the maintenance medium at the end of the viral absorption period, while specifically neutralizing hemagglutinin and hemadsorption resulting from the infection in each case, failed to affect the development of fusion resistance. This suggested that fusion resistance was not the result of receptor destruction by newly released virions. Accordingly, in separate experiments, FL cells were treated with BPL-inactivated Pfl 2 at a concentration of hemagglutinin equivalent to that produced by an actively infected cell sheet. The BPL inactivated virions retained full enzyme activity. The treated cells remained fusible, and also were susceptible to infection with either active Pfl2 or Sendai virus. This constituted further evidence that receptors remained intact and hence that neraminidase activity of free or released virions was not sufficient to affect the development of refractoriness to fusion.

In monolayers of FL cells infected with either measles or RS virus, the cytopathic effect was characterized by the formation of moderately large syncytia, which, along with a high proportion of mononucleated cells, contained viral antigen revealed by fluores-

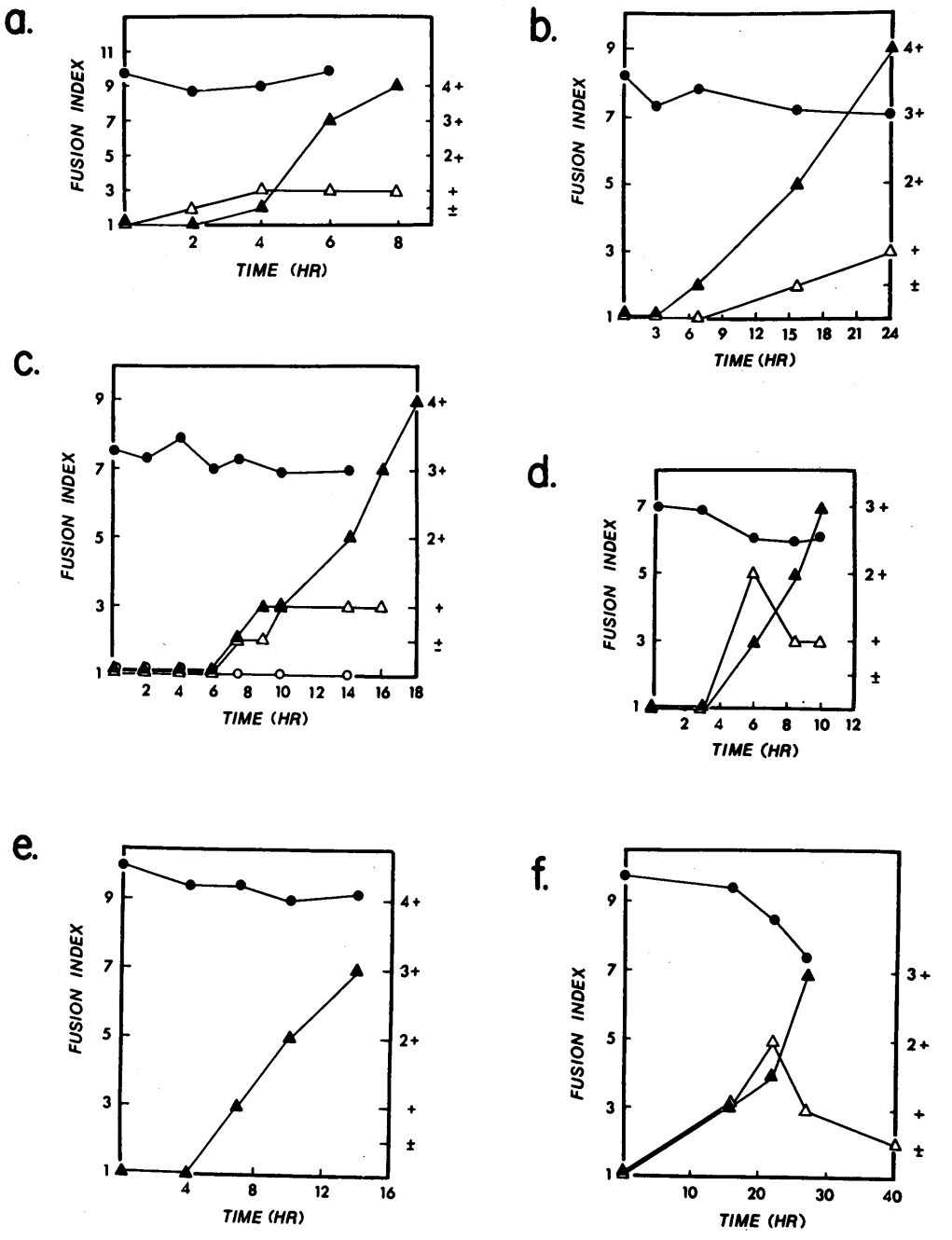
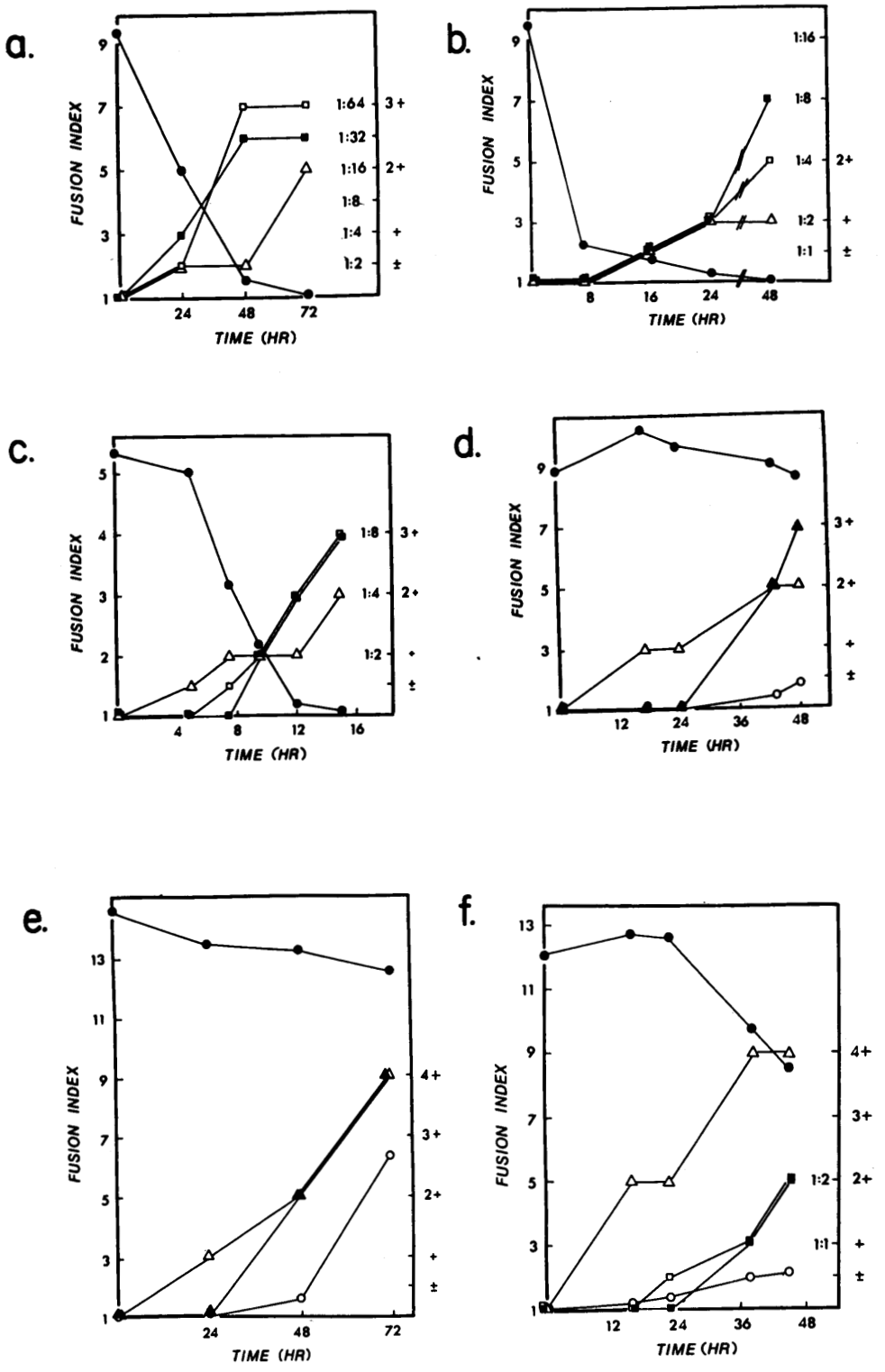


FIG. 1. Susceptibility of FL amnion cells to exogenous fusion by inactive Sendai virus (400–800 HAU) at various times after infection with (a) poliovirus, type 1; (b) adenovirus, type 7; (c) herpes simplex virus; (d) vesicular stomatitis virus; (e) vaccinia virus; and (f) Sindbis virus. ● = Fusion index; ▲ = Cytopathic effect (0–4+); △ = Specific fluorescence, primary infection (0–4+).


a.
FUSION INDEX
TIME (HR)
1:64
1:32
1:16
1:8
1:4
1:2
3+
2+
+
±
b.
FUSION INDEX
TIME (HR)
1:16
1:8
1:4
1:2
1:1
2+
+
±
c.
FUSION INDEX
TIME (HR)
1:8
1:4
1:2
3+
2+
+
±
d.
FUSION INDEX
TIME (HR)
3+
2+
+
±
e.
FUSION INDEX
TIME (HR)
4+
3+
2+
+
±
f.
FUSION INDEX
TIME (HR)
1:2
1:1
4+
3+
2+
+
±

cence microscopy. These heavily infected monolayers showed undiminished susceptibility to extrinsic fusion (Fig. 2, d–f), challenge with inactive Sendai virus causing the massive coalescence of all elements into a single sheet of nuclei in which remnants of pseudomyxoviral syncytia were readily discernible (Fig. 3).

Discussion. These experiments were designed to determine whether susceptibility of cells in monolayers to Sendai virus fusion could be altered as a result of prior infection with any of a number of representative viruses, some of which are recognized as having profound effects on the cell membrane. In all instances, there was close to 100% infection by the primary virus, as judged by immunofluorescence microscopy of control infected monolayers. As recently shown by others (6), vaccinia-infected cells retained undiminished susceptibility to exogenous Sendai virus fusion. The same was true of cells infected with HSV, adenovirus or poliovirus, presumably because none of these agents involves the plasma membrane directly in viral maturation and release. However, it has been shown that mature Sindbis virus bears glycoprotein derived from the walls of the cytoplasmic vesicles (7) and possibly from polysaccharide components of the cell membrane. Likewise, direct involvement of the plasma membrane in VSV development and maturation has been demonstrated (8–10) and it has been shown that glycolipids in the viral envelope are derived from the host cell plasma membrane (11). However, preinfection of cells with either Sindbis virus or VSV had no inhibitory effect on subsequent extrinsic Sendai fusion, implying that the host-derived glycoproteins of the virus in neither case include myxovirus receptors.

Maturation of myxoviruses and paramyxoviruses at the plasma membrane is associated with striking morphological and antigenic alterations of segments of the plasma membrane from which virions ultimately bud. These altered segments are found to contain antigens characteristic of the viral envelope, namely, hemagglutinin and neuraminidase (12–14). It has also been shown that lipids and glycolipids of the mature virions are derived from the plasma membrane (15–17). In attempting to explain the resistance to extrinsic fusion which develops during maturation of any of these myxoviruses, one can suppose that newly synthesized viral neuraminidase acts at the plasma membrane to cleave sialic acid from adjacent receptor sites. Initial attachment of extrinsic, fusing virus is thereby prevented, and hence, polykaryon formation does not occur. In the case of both influenza and Pfl 2 viruses, the first expression of refractoriness occurred concomitantly with the detection of new viral hemagglutinin in the medium and hemadsorption. Our data support the contention that cell-bound neuraminidase, inaccessible to antibody, was primarily responsible for receptor destruction, and that neuraminidase on released particles was not involved. Fusion resistance developed as well in the presence of viral antibody as in its absence. The refractory state developed much more rapidly with type 2 parainfluenza virus than with either influenza or Sendai viruses. This may be explained by the greater efficiency with which Pfl 2 develops in FL cells compared to either PR8 or Sendai viruses, both of which produce only an abortive infection in this system.

The two pseudomyxoviruses studied, measles and RS viruses, although developing in a manner quite similar to that of the true myxoviruses, both lack neuraminidase. Apparently, the distortions in the cell membrane associated with the maturation and release of either one of these agents, although marked,

FIG. 2. Susceptibility of FL amnion (a–d, f) or HEp-2 cells (e) to exogenous fusion by inactive Sendai virus (400–800 HAU) at various times after infection with (a) influenza virus (PR8); (b) type 1 parainfluenza (Sendai) virus; (c) type 2 parainfluenza virus; (d) respiratory syncytial virus (FL cells); (e) respiratory syncytial virus (HEp-2 cells); and (f) measles virus. ● = Fusion index; △ = Specific fluorescence, primary virus (0-4+); ■ = Hemagglutination titer; □ = Hemadsorption (0–4+); ○ = Syncytia formed by primary virus (d, e, f).

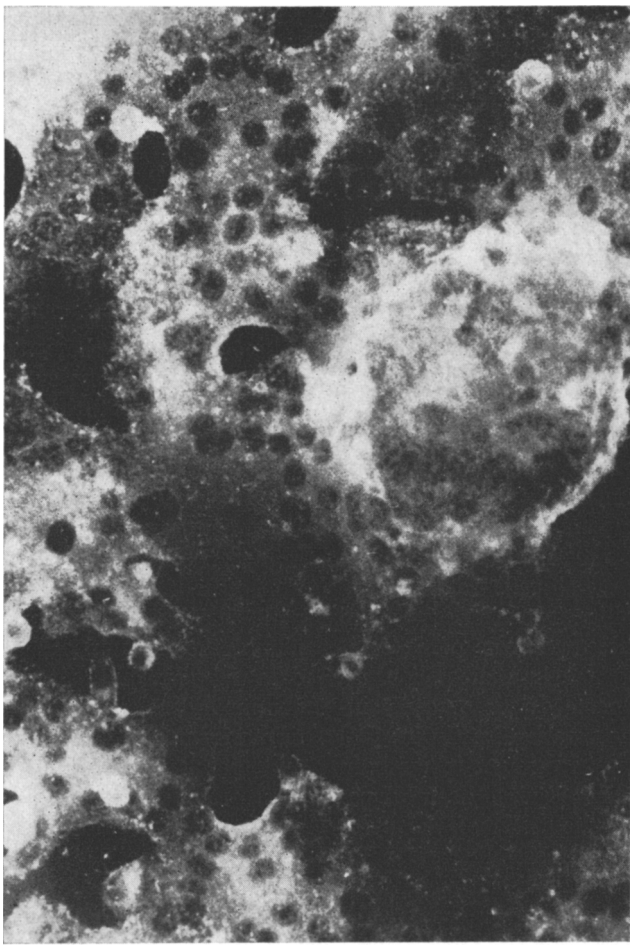


FIG. 3. HEp-2 cells 48 hr after infection with respiratory syncytial virus, fused by exogenous inactive Sendai virus and stained with fluorescein-conjugated anti-RS virus globulin.

are not great enough to affect the susceptibility of the cells to fusion by Sendai virus. These findings are also consistent with previous observations establishing that the cell receptors, for both infection and hemagglutination by measles virus, differ fundamentally from receptors for myxoviruses and do not involve sialoglycoprotein residues (18).

Summary. The development of resistance to extrinsic fusion by Sendai virus in cells infected with myxoviruses or paramyxoviruses can be attributed to the destruction of sialoglycoprotein receptors by newly synthesized viral neuraminidase incorporated in the cell membrane and inaccessible to antibody. Infection with any of the viruses which lack neuraminidase does not influence susceptibility to extrinsic fusion by Sendai virus, even though the cell membrane may undergo specific chemical and morphological alterations during viral maturation and release.

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