The Functions and Inhibition of the Membrane Glycoproteins of Paramyxoviruses and Myxoviruses and the Role of the Measles Virus M Protein in Subacute Sclerosing Panencephalitis

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The F glycoprotein of paramyxoviruses is responsible for cell fusion and hemolysis and for virus penetration via fusion of viral and cell membranes. These functions are activated by specific proteolytic cleavage of an inactive precursor (F₀) into two disulfide-linked polypeptides (F₁ and F₂). The susceptibility of the F₀ protein to cleavage by a host protease is a major determinant of virus host range and virulence. Synthetic oligopeptides that mimic the N-terminal region of the F₁ polypeptide are specific inhibitors of paramyxoviruses, and oligopeptides that mimic the N-terminus of the HA₂ polypeptide of influenza virus, also generated by cleavage, specifically inhibit that virus. Antibodies to F protein prevent the spread of paramyxovirus infection via membrane fusion, but antibodies to HN protein do not, although they neutralize released virus. These results and previous findings that formalin-treated virus does not induce antibodies to F protein provide an explanation for atypical measles. The HN protein has both receptor-binding and neuraminidase activities, and Cl⁻ inhibition of neuraminidase may modulate these antagonistic activities. Studies in patients with subacute sclerosing panencephalitis (SSPE) suggest that there is a host restriction of synthesis of the M protein of measles virus in brain cells which is involved in the abortive, persistent infection that causes SSPE.

The myxoviruses (influenza viruses) and paramyxoviruses are the etiological agents of many economically important diseases of humans and other animals. Influenza continues to occur in epidemics and occasional pandemics, and it ranks among the leading causes of morbidity and mortality throughout the world. The large group of paramyxoviruses, which includes parainfluenza 1–5, mumps, measles, canine distemper, and Newcastle disease viruses, causes a variety of illnesses ranging from acute respiratory to chronic neurological diseases. Our laboratory has been engaged for many years in studies of the structure, replication, and mechanisms of pathogenesis of these viruses, with emphasis on the viral membrane and the biological activities of the viral proteins. These investigations have been carried out not only because of the importance of the diseases caused by these viruses, but also because they serve as excellent models for studies of the structure and biogenesis of cell membranes [1]. Viral membranes are useful in this regard because they are assembled by the same process as the plasma membrane of the host cell; they contain only a few proteins, which can be isolated and purified; they can be studied using mutants, thus bringing to bear the power of the genetic approach; and the protein, lipid, and carbohydrate content of the viral membrane can be varied independently by selecting the appropriate virus strain, cell type, and growth medium. This paper describes studies on the structure and function of the membrane proteins of paramyxoviruses and, to a lesser extent, myxoviruses; on the role of these proteins in viral pathogenesis; and on the specific inhibition of the functions of the proteins. These studies have been described in a number of primary publications, and many of the results were reviewed in 1980 [2]; therefore, they will be summarized briefly here.

Structure and function of paramyxovirus membrane glycoproteins. The envelope of paramyxoviruses consists of a lipid bilayer, which is derived...
from the host-cell plasma membrane during virus assembly by budding, and three virus-coded proteins [3]. A nonglycosylated protein—designated M—is associated with the inner surface of the lipid bilayer, and two glycoproteins—designated HN and F—from the outer surface of the viral membrane. The HN and F glycoproteins have been isolated and purified in biologically active form using nonionic detergents and various chromatographic, precipitation, and sedimentation methods [4-10]. These proteins form spike-like projections \( \sim 10 \, \text{nm} \) long on the surface of the virion and are anchored in the lipid bilayer of the viral membrane by a hydrophobic region at the C-terminal end of the protein.

The HN glycoprotein has a relative molecular weight (Mr) of \( \sim 65,000-70,000 \), depending on the virus strain. In most paramyxoviruses the HN protein has both receptor-binding (hemagglutinating) activity and neuraminidase activity, which is capable of destroying the neuraminic acid-containing receptors for the virus [4, 5]. In the morbillivirus subgroup (measles, canine distemper, and rinderpest viruses), the analogous protein lacks neuraminidase activity and is designated H. The receptor for morbilliviruses does not contain neuraminic acid, but the exact nature of this receptor is unknown.

The HN glycoprotein is responsible for the first step in infection, the adsorption of the virus to receptors on the host cell. The presence of both receptor-binding and receptor-destroying activities on the same viral glycoprotein presents an apparent paradox with respect to the initiation of infection. We have recently obtained evidence that may provide an explanation [11]. The neuraminidase activity of several paramyxoviruses was found to be inhibited by concentrations of halides, including Cl\(^{-}\), in concentrations found in extracellular fluids under physiological conditions. In contrast to the inhibition of neuraminidase activity, halides enhanced hemagglutinating activity and decreased virus elution from erythrocytes, and the greater the inhibition of neuraminidase, the higher the hemagglutination titer. Furthermore, the pH optimum of the neuraminidases of the several paramyxoviruses studied is acidic. The observed reciprocal effects on receptor binding and receptor destruction suggest that these two opposing activities of the HN glycoprotein can be regulated by environmental conditions such as Cl\(^{-}\) concentration and pH. Thus, virus attachment would be favored by the high concentration of Cl\(^{-}\) and the neutral pH found in the extracellular environment, whereas neuraminidase activity would be favored by the lower intracellular concentration of Cl\(^{-}\) and pH. On the other hand, these results [11] and our review of the literature suggest that the viral neuraminidase activity, which is important for the release and dissemination of maturing virions, is expressed before the arrival of viral glycoproteins and glycolipids at the external surface of the plasma membrane.

Studies of the structure of the HN protein have revealed that it is present on the surface of the virion as a dimer that is held together by both disulfide bonds in its hydrophilic region and hydrophobic bonds at the base of the protein [10]. Under appropriate conditions, the hydrophobic base (\( \sim 5,000 \) daltons) of the HN protein of the parainfluenza virus SV5 (simian virus) can be removed by a proteolytic enzyme, yielding a water-soluble protein (Mr, \( \sim 59,000 \)) which retains receptor-binding and neuraminidase activities, although it cannot function as an agglutinin because it is monovalent [10]. Isolated intact HN molecules aggregate by their hydrophobic bases when detergent is removed, forming rosette-like clusters which thus are multivalent and hemagglutinate [4].

Because neuraminic acid-containing macromolecules that can act as receptors for paramyxoviruses or myxoviruses are ubiquitous on the surfaces of vertebrate cells, the adsorption of these viruses to receptors is not a step in replication that plays an important role in determining host range or tissue tropism, in contrast to the situation with some other viruses. For example, with picornaviruses [12] virus-receptor interactions play a decisive role in host-cell specificity. Thus, the HN protein of paramyxoviruses, although it mediates the important step of virus adsorption, is not a significant factor in determining host range and tropism. In contrast, the other viral surface protein, F, plays a crucial role in this regard.

The F glycoprotein of paramyxoviruses is synthesized as a biologically inactive precursor (Fo) which has an Mr of \( \sim 65,000 \). Under appropriate conditions, the Fo moiety is cleaved by a host-cell protease to yield two disulfide-bonded polypeptides (F\(_{1}\) and F\(_{2}\)) with an Mr of \( \sim 50,000 \) and \( \sim 15,000 \), respectively [6, 13-15]. The F\(_{1}\) polypeptide contains the C-terminal region of the precur-
or which is anchored in the viral membrane, and the F₂ polypeptide contains the original N-terminus [15]. The F protein is responsible for several important biological activities, all of which involve membrane fusion: penetration of the virus into the host cell through fusion of the viral and cell membranes, cell fusion, and hemolysis. Unlike the isolated HN protein, the isolated F protein has no biological activity either as a pure protein or if it is incorporated alone into a lipid vesicle. Because the activities of the F protein involve the fusion of membranes, the F protein must not only be incorporated into a membrane to act, but it must also be delivered to the target cell membrane, a function supplied in the virus by the HN protein. We have shown [9] that pure F protein, when it is reconstituted into a membrane with lipids, is biologically active if a means of attachment of the reconstituted membrane to the cell is provided. Such attachment can be achieved using either the viral HN protein or a lectin (for example, wheat germ agglutinin).

Although such studies with isolated F protein demonstrate its activity, the initial evidence for the function of the F protein came from studies showing that some cells produced virus that contained the uncleaved F₀ precursor. This virus was not infectious and lacked hemolyzing and cell-fusing activities. Cleavage of the F₀ moiety on these virus particles to yield the F₁ and F₂ fragments activated the infectivity of the virus and the virus-induced properties of cell fusion and hemolysis [6, 13–15]. This activating proteolytic cleavage is normally accomplished by a host enzyme, and some cells lack the appropriate enzyme to cleave the protein of certain viruses. For example, Madin-Darby bovine kidney cells or mouse fibroblasts (L cells) do not express an enzymatic activity capable of cleaving the Sendai virus F₀ precursor and therefore produce inactive virions, which can be activated in vitro by trypsin [6, 13, 14]. The specificity of this reaction has been clearly illustrated by the isolation of mutants that require different proteases from wild-type viruses for activation and which exhibit a different host range at levels of both cultured cells and the chicken embryo [14].

The role of paramyxovirus F protein in tropism and pathogenesis. The finding that the ability of the virus to initiate infection and to undergo multiple-cycle replication depends on the cleavage of the F protein by a host enzyme indicated that the host range and tissue tropism of the virus and its ability to spread in the host and ultimately to cause disease are dependent on the availability of the appropriate activating protease in the host [6, 14–17]. On the basis of these results in cultured cells and chicken embryos, we postulated [17] that the pathogenesis of paramyxoviruses in their natural hosts might also depend on the susceptibility of the viral F protein to cleavage by host enzymes, citing as possible examples virulent and avirulent strains of Newcastle disease virus. The role of proteolytic cleavage in viral virulence in animals was subsequently clearly demonstrated in chickens by Nagai et al. [18] and Nagai and Klenk [19], who examined many strains of Newcastle disease virus and found that virulence correlated with the ability of chicken fibroblasts to cleave the viral F protein.

The studies described above have clearly shown that the F protein plays a central role in the pathogenesis of paramyxoviruses by influencing tropism and the ability of the virus to undergo multiple-cycle replication, to spread in the host, and thus to cause disease. In addition to this role of the F protein, it has been recognized for many years that cell fusion, now known to be mediated by the F protein, is a major cause of CPE in paramyxovirus infections. In some systems it has been demonstrated that whether a paramyxovirus-infected cell survives or dies depends on whether cell fusion occurs [20–22]. As will be discussed below with regard to atypical measles, the F protein of paramyxoviruses may also be involved in viral pathogenesis by yet another mechanism, an immunopathological reaction.

Implications of cleavage of F protein for virus isolation, replication in cultured cells, and attenuation. In addition to elucidating the mechanism of virus penetration and virus-induced cell fusion and demonstrating the role of the F protein in pathogenesis, the discovery of the proteolytic activation of the F protein has other implications. It has been frequently observed in the past that although primary cultures of cells such as rhesus monkey kidney cells support the replication of a virus, serial passage of the cells is accompanied by a rapid and progressive decline in their ability to do so. Using Sendai virus and rhesus monkey kidney cells as a model system, we found that this de-
cline is due to a progressive loss in the activating protease by the cells [23]. Another important consequence of the requirement of cleavage for virus replication is that whenever a virus is isolated from an infected individual, the F protein of that virus must be susceptible to cleavage by an enzyme present in the particular host cell or animal used in the isolation, and it is possible that a virus will be selected in the isolation procedure that does not represent the major virus population present and causing disease. Thus, by passage in a new host, virus variants may be selected whose glycoproteins are cleaved by a protease present in the laboratory host but that are less susceptible to cleavage by enzymes available in the cells of the human or other natural host. Such variant selection may provide an explanation for some instances of rapid attenuation of viruses by a single passage or a few passages in cultured cells or in laboratory animals.

Structure-function relationships of F protein. Because of the biological importance of the cleavage of the F protein, further investigation of the structure of the protein was undertaken with emphasis on the region around the cleavage site. The cleavage generates a new N-terminus on the F\(_1\) polypeptide [15], and several lines of evidence suggested that this new N-terminus was involved in the biological activities of the protein. The first suggestion of this function was the initial finding that the expression of these activities is dependent on the cleavage. The amino acid sequences beginning at the N-terminus of the F\(_1\) polypeptides subsequently were determined for three different paramyxoviruses with different natural hosts: SV5, Newcastle disease virus, and Sendai virus [10, 23, 24]. The sequences of the first 18 amino acids at the N-termini of the F\(_1\) polypeptides of these viruses are shown in figure 1.

There is a very high degree of homology in these sequences from three viruses with different natural hosts. Thus, the primary structure of this region of the protein is highly conserved. The biological importance of a specific amino acid sequence in this region of the polypeptide was also indicated by the finding that a mutant of Sendai virus that is activated by a different protease than that which activates wild-type virus had the same N-terminal sequence as the wild-type virus: Phe-Phe-Gly. Another striking feature of this region of the polypeptide shown by the sequences in figure 1 is that it is extremely hydrophobic, a property which raised the possibility that it could be involved in hydrophobic interactions with the target cell membrane. We also noted a similarity between these N-terminal sequences of the F\(_1\) polypeptide and an oligopeptide [carbobenzoxy-d-Phe-l-Phe-l-(NO\(_2\))-Arg] that had been found earlier to inhibit plaque formation, hemolysis, and cell fusion by measles virus [22, 25, 26] and by SV5 (P. W. Choppin, unpublished observations).

Specific inhibition of activities of F glycoprotein. The results summarized above strongly suggested that the N-terminal region of the F\(_1\) polypeptide of paramyxoviruses was involved in the biological activities of the F protein, and we therefore thought that it might be possible to inhibit these activities specifically with oligopeptides that resembled this N-terminus. Many oligopeptides were therefore synthesized, using the N-terminal region of the Sendai virus F\(_1\) polypeptide as the primary model, but with variations in the sequence and steric configuration of the amino acids and substitutions at the N- and C-termini. These oligopeptides were tested against Sendai and measles viruses and SV5. Oligopeptides with the correct composition could inhibit virus infectivity, virus-induced cell fusion, and hemolysis. However, in most experiments the inhibition of infectivity at the level of virus penetration was determined using plaque formation as the assay because of the greater sensitivity of this method. In each experiment, several concentrations of each polypeptide were tested for inhibition of plaque formation, and dose-response curves were obtained [27].

Sendai and measles viruses and SV5 were each inhibited by the appropriate oligopeptides; how-

![Table 1](https://academic.oup.com/jid/article-abstract/143/3/352/863018)
Table 1. Inhibition by various oligopeptides of plaque formation by measles virus.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>50% inhibitory concentration (μM)</th>
</tr>
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<tbody>
<tr>
<td>Z-D-Phe-L-Phe-Gly</td>
<td>0.02</td>
</tr>
<tr>
<td>Z-D-Phe-L-Phe-Gly(methyl ester)</td>
<td>0.20</td>
</tr>
<tr>
<td>Z-L-Phe-L-Phe-Gly</td>
<td>20</td>
</tr>
<tr>
<td>Z-L-Phe-L-Phe-Gly(methyl ester)</td>
<td>23</td>
</tr>
<tr>
<td>Z-D-Phe-L-Phe</td>
<td>28</td>
</tr>
<tr>
<td>Z-L-Phe-L-Phe</td>
<td>42</td>
</tr>
<tr>
<td>Z-L-Phe-L-Ser</td>
<td>141</td>
</tr>
<tr>
<td>Z-Gly-L-Phe-L-Phe</td>
<td>530</td>
</tr>
<tr>
<td>D-Phe-L-Phe-Gly</td>
<td>180</td>
</tr>
</tbody>
</table>

NOTE: Data are from Richardson et al. [27]. Z = carbobenzoxyl.

However, measles virus was the most sensitive to their action. For example, the 50% inhibitory concentrations of the tripeptide Z-Phe-Phe-Gly against SV5 and Sendai and measles viruses were 400 μM, 320 μM, and 0.2 μM, respectively. (Z designates the carbobenzoxyl group.) Because of the greater sensitivity of measles virus, detailed structure-activity studies were carried out with this virus [24]. Oligopeptides with the appropriate amino acid sequence were highly active, specific inhibitors. The results of extensive structure-activity studies [24] are summarized briefly below, with examples shown in table 1.

The inhibition by the oligopeptides is amino acid sequence-specific, and the presence of the same amino acid on the oligopeptide as the viral polypeptide is crucial for activity: Gly-Phe-Phe is much less active than Phe-Phe-Gly (table 1). If the oligopeptides have the correct sequence, longer peptides are more active than shorter ones with the same initial sequence. The most active inhibitor tested thus far (50% inhibitory concentration, 0.02 μM) is a heptapeptide with the same sequence as the first seven amino acids found in Sendai virus. The amino acid–sequence specificity of the inhibition was also strikingly demonstrated using a mutant of measles virus that had been selected by passage in the presence of a tripeptide; the mutant lost its sensitivity to that peptide but remained sensitive to another tripeptide that differed only in the third amino acid.

The presence of the Z group on the N-terminal amino acid of an oligopeptide increases inhibitory activity, and the addition of a methyl group to the carboxyl moiety of the C-terminal amino acid decreases activity (table 1). The reason why the addition of these groups has a significant effect on inhibitory activity is not known; however, they may be involved in the positioning of the inhibitor at its site of action, that is, the Z group provides additional hydrophobicity at the N-terminus, and esterification of the C-terminal amino acid decreases the polarity of the peptide. These changes could therefore affect the orientation of the peptide.

The steric configuration of the N-terminal amino acid of the oligopeptide inhibitors is also important; greater activity was obtained with D-phenylalanine as the N-terminal amino acid than with the naturally occurring L-phenylalanine (for example, Z-D-Phe-L-Phe-Gly and Z-L-Phe-L-Phe-Gly [table 1]). It is possible that this increase in activity might be related to protection by the D-amino acid from proteolytic degradation in the assay system; however, it is also possible that a steric effect is directly involved in inhibitory activity. The D-phenylalanine-containing tripeptide was also more active in inhibiting hemolysis, an assay in which proteolytic activity is less likely to be a factor than in plaque formation. It is clear that D-amino acid-containing peptides do not act by simply inhibiting the metabolic activity of the cell. Not one of the oligopeptides tested appears to be toxic in the system studied; cells survive for many days in the presence of the peptides, exhibit no detectable CPE, and multiply normally.

Our studies have thus shown that oligopeptides with amino acid sequences that resemble the N-terminal region of the F1 polypeptide of paramyxoviruses are highly active, specific inhibitors of viral infectivity and of virus-induced cell fusion and hemolysis, activities which involve fusion of viral and cell membranes. Although the precise mechanism of action is not yet clear, the results suggest that the oligopeptides in some way competively interfere with the N-terminal region of the F1 polypeptide. Oligopeptides have been synthesized with both radioactive (3H) and fluorescence (dansyl) labels. The use of these oligopeptides should permit the determination of the site and mechanism of action of these inhibitors and also provide more information on the mechanism by which the F protein induces membrane fusion and initiates infection. In addition, further studies in cultured cells and in animals should reveal whether such inhibitors provide a useful new approach to the control of certain viral infections.
Specific inhibition of influenza virus replication by oligopeptides. The influenza viruses also possess two glycoproteins on their surface: the hemagglutinin (HA) and neuraminidase (NA) proteins. However, unlike in the paramyxoviruses, the receptor-binding (hemagglutinating) and neuraminidase functions reside in two different proteins (HA and NA, respectively). In 1971 we found that the HA glycoprotein is cleaved by a host protease [28]. This cleavage, which can be accomplished by a cellular enzyme or plasmin in serum [28-30], yields two disulfide-bonded polypeptides (HA1 and HA2), and a new N-terminus is generated on the HA polypeptide, the C-terminus of which is embedded in the viral membrane [28, 30-32]. Although the cleavage of the HA protein does not affect hemagglutinating activity, it activates the infectivity of the virus, presumably at the level of penetration [33, 34]. Furthermore, as in the paramyxoviruses, there is a correlation between the virulence of influenza viruses in the chicken and the cleavability of the HA protein by avian cell proteases [35]. Thus, as pointed out [10], there is a structural and functional analogy between the F protein of paramyxoviruses and the HA protein of myxoviruses; both are involved in the initiation of infection and are activated by cleavage by a host protease to yield two disulfide-bonded polypeptides. In addition, the sequence of the first nine amino acids at the new N-terminus generated on the HA2 polypeptide of influenza viruses resembles that of the N-terminus of the F1 polypeptide of paramyxoviruses, except that on HA2 there is an N-terminal glycine that precedes phenylalanine, which is the N-terminus of the F1 polypeptide of paramyxoviruses. Skehel and Waterfield [32] found the amino acid sequence of Gly-Phe-Phe-Gly-Ala-Ile-Ala-Gly-Phe-Leu for the Lee strain of influenza B virus. For several strains of influenza A virus, the same sequence—except that leucine was substituted for the first phenylalanine and isoleucine for the terminal leucine—was found. Subsequently many strains of influenza virus have been examined, and the N-terminal sequence of HA2 is the most highly conserved region on the influenza virus HA molecule [36-38]. The similarity of this region to the N-terminus of the F1 polypeptide of paramyxoviruses can be seen by comparing the N-terminal sequence of influenza B virus HA2 with those of the paramyxoviruses given in figure 1.

Because of the structural and functional similarities between the HA2 and F1 polypeptides of the two groups of viruses and because of the success we had had with oligopeptide inhibitors of paramyxoviruses, oligopeptides were synthesized that resembled the N-terminus of the influenza virus HA2 polypeptide and were tested for their ability to inhibit viral replication. With influenza virus as well, there was amino acid sequence–specific inhibition by these oligopeptides. Z-Gly-L-Leu-L-Phe-Gly and Z-Gly-L-Phe-L-Phe-Gly, which resemble the N-terminal sequences of influenza A and B viruses, respectively, were highly active; the 50% inhibitory concentration of the former oligopeptide for the WSN strain of influenza A virus was 20 μM. However, Z-D-Phe-L-Phe-Gly, which resembles the paramyxovirus sequence, was much less active against influenza virus (50% inhibitory concentration, 290 μM). The reverse was true with paramyxoviruses (table 1). Thus, as with influenza virus, there is amino acid sequence–specific inhibition, with the correct N-terminal amino acid being very important for optimal activity. These findings emphasize the biological importance of this region of the HA2 polypeptide and the functional relatedness of the HA and F proteins of the two different groups of viruses. The results also support the concept that the activation of the infectivity of influenza virus by proteolytic cleavage involves the penetration step and that fusion between viral and cell membranes is involved in penetration, as it is with the paramyxoviruses. Further studies with specific oligopeptide inhibitors should provide additional knowledge of the early interactions of both groups of viruses with cell membranes and reveal whether the use of such inhibitors specifically designed to mimic regions of viral proteins holds any promise as a new approach to the chemoprophylaxis or chemotherapy for infections with influenza and parainfluenza viruses.

The importance of immunity to F glycoprotein in prevention of spread of paramyxovirus infection. It has long been known that the surface glycoproteins of enveloped viruses are involved in the generation of immunity to infection. However, the determination of the importance of the individual paramyxovirus glycoproteins required the use of specific antibodies to each protein and an examination of the effectiveness of these antibodies in preventing not only the initiation of in-
fection but also the spread of infection. Therefore, we prepared antibodies to each of the envelope proteins of a parainfluenza virus (SV5) and investigated their inhibitory activities [39, 40]. As expected from the previous studies of the activities of the isolated proteins discussed above, antibodies to the HN protein inhibited both hemagglutinating and neuraminidase activities. Antibodies to the HN protein also neutralized virus infectivity by interfering with adsorption when a conventional neutralization test was done in which virus and antibodies were mixed before inoculation of cells. Similar results had been obtained with Newcastle disease virus [41]. Antibodies to the F protein of SV5 inhibited cell-fusing and hemolyzing activities and neutralized infectivity in a conventional neutralization test by interfering with viral penetration. However, significant results were obtained when the ability of antibodies to the two proteins to inhibit the spread of infection from cell to cell was investigated [39]. In these experiments antibodies to the individual glycoproteins were added to a monolayer of cells after a few cells had been infected by inoculation at a low multiplicity of infection. These conditions should resemble more closely than a conventional neutralization test the conditions in an individual whose respiratory epithelium has been seeded with an inoculum of virus. In a population of cells, a paramyxovirus can spread either by released virus adsorbing to and infecting other cells or by fusion of the membrane of an infected cell with that of an adjacent cell as a result of the activity of the F protein. In experiments with antibodies specific to each protein [39], we found that whereas antibodies to the HN protein inhibited the dissemination of infection by released virus, they did not prevent the spread of infection by membrane fusion. Thus, in cells that were susceptible to the fusing activity of the F protein, infection spread from cell to cell in the presence of antibodies to the HN protein. In contrast, antibodies to the F protein completely prevented the spread of infection because they were capable not only of neutralizing released virus but also of inhibiting the activity of the F protein and thereby preventing cell-to-cell spread by membrane fusion. These results have indicated that effective immunological prevention of the spread of paramyxovirus infections must involve inactivation of the F protein, and thus an effective vaccine must induce antibodies to this protein.

Lack of antibodies to F protein and atypical measles. The above findings suggest an explanation for the early failures of formalin-killed paramyxovirus vaccines and for the atypical and severe infections that have been observed in individuals who received killed measles virus or respiratory syncytial virus vaccines and were subsequently infected with the virus [42, 43]. Norrby et al. [44], Norrby and Gollmar [45], and Norrby and Penttinen [46] found that formalin- or Tween-ether-inactivated measles and mumps virus vaccines induced hemagglutinating-inhibiting, but not hemolysis-inhibiting, antibodies (the latter are now known to be antibodies to the F protein) and suggested that the atypical measles which occurred in individuals receiving such vaccines might be related to the lack of these antibodies. Other investigators have pointed out that the atypical measles and the severe respiratory syncytial infections in vaccinees had several features of an immunopathological process [43, 47, 48].

Correlation of our findings that antibodies to the F protein are required to prevent completely the spread of paramyxovirus infections with the previously observed lack of antibodies to the F protein in individuals receiving the vaccines and with the clinical findings suggesting an immunopathological reaction led us to propose the following explanation for the atypical infections [39]. The killed vaccines induced antibodies to the HN (or H in the case of measles) protein but not the F protein. When such individuals were subsequently exposed to the virus, some cells in the respiratory tract were infected, and the infection spread to adjacent cells by fusion because of the lack of antibodies to the F protein. As infection spread in this manner, viral antigens were produced and released; these viral antigens served as secondary antigenic stimuli, resulting in a hyperimmune response to H and the other viral proteins. Recent studies have shown that the convalescent-phase sera of patients with atypical measles contain high levels of antibodies to measles virus, not only to the H protein but also to other viral proteins [49] (W. W. Hall, M. H. Kaplan, and P. W. Choppin, unpublished observations). Thus, a situation appears to exist in these patients in which viral replication and spread by fusion occur, providing a continuing source of antigens at the same time that a secondary immune response is being mounted to the antigens being produced. This sequence of events may ex-
plain the findings in atypical measles and possibly in severe infections due to respiratory syncytial virus, although it is not yet known which protein of respiratory syncytial virus is involved in cell fusion or whether formalin inactivates the antigenicity of that protein, as it does in the case of the other paramyxovirus F proteins.

Because of the early failures of formalin-killed paramyxovirus vaccines, there was an understandable loss of interest in the possibility of developing inactivated vaccines for these viruses. However, now that it is clear that antibodies to the F protein are essential for an effective and safe vaccine, this question should be reevaluated, in particular because methods are available for isolating the proteins in biologically active form [4, 7, 9, 40, 50]. The use of isolated proteins would have the dual advantages of avoiding the use of formalin and providing a pure preparation of only the proteins desired. Vaccines consisting of isolated purified glycoproteins, alone or incorporated into lipid-containing vesicles [9] to increase the size and possibly the antigenicity of the preparation, could represent important additions to our immunization possibilities against important diseases caused by paramyxoviruses. The potential use of recombinant DNA techniques to enable the production of viral proteins in bacteria provides another stimulus for reconsideration of the use of isolated paramyxovirus proteins as vaccines.

The Nonglycosylated Membrane Protein of Measles Virus and Subacute Sclerosing Panencephalitis

**The M protein of paramyxoviruses.** In addition to the HN and F glycoproteins on the surface of paramyxoviruses, there is a nonglycosylated protein (M) associated with the viral envelope [51, 52]. The M protein is associated with the inner surface of the lipid bilayer and plays a key role in the assembly of the virus particle by budding from the plasma membrane [3, 53]. It interacts on the one hand with the glycoproteins that are inserted in the cell membrane and on the other hand with the viral nucleocapsid, serving as the recognition site for the nucleocapsid as it aligns beneath the areas of the cell membrane that contain viral envelope proteins and are destined to become the viral envelope [3, 53, 54]. Recent studies from our laboratory [49, 55–58] have suggested that an abnormality in the synthesis of the M protein of measles virus is involved in the pathogenesis of subacute sclerosing panencephalitis (SSPE).

**SSPE.** An important biological characteristic of paramyxoviruses is their ability to cause persistent infections both in cultured cells and in vivo [3]. An important example of such an infection is SSPE, a fatal chronic neurological disease caused by a persistent, abortive infection with measles virus [59–62]. Several hypotheses to explain the occurrence of SSPE have been proposed in the 15 years since the first evidence was uncovered for the involvement of measles virus in the disease. With the increasing knowledge of the measles virus particle, its replication, and its effects on cells, it has been possible to gain some insight into the mechanisms involved in the pathogenesis of SSPE. The existence of a specific immunological deficit in the patients has been entertained, but work in several laboratories has failed to substantiate such a defect. A possibility that has received considerable attention is that a mutant or variant of measles virus is responsible for the disease, and there have been reports from a number of laboratories describing some differences between some wild-type measles and SSPE strains, including differences in growth rate or CPE, in the electrophoretic mobility of viral proteins or messenger RNAs, in antigenicity of certain proteins, and in the complexity of viral genome RNAs. Although some differences have been noted between certain strains, they are not consistent when other strains are examined, and at present there is no biochemical or biological marker that will unequivocally differentiate an SSPE strain from a wild-type measles virus strain. It should be emphasized that such findings do not exclude the possibility that SSPE strains are mutants of wild-type measles viruses, but the existence of specific SSPE-causing mutants has certainly not been established. An important point in considering the mutant virus hypothesis is that the epidemiology of SSPE suggests that "SSPE strains" are not circulating in the population; there are no clusters of cases to suggest that a certain group of people was infected with an SSPE strain, in contrast to a wild-type virus that infects the majority of the population. The evidence therefore suggests that if a mutant virus is the cause of SSPE, that mutant must arise independently in each patient during the course of infection. Because the disease is so rare (about one case of
SSPE per million cases of measles), such an event, though unlikely, is not impossible.

**Lack of antibodies to M protein in SSPE.** Recently evidence has been obtained that suggests another factor in the pathogenesis of SSPE. Following the initial work of Connolly et al. [60], studies in many laboratories established that patients with SSPE had high titers of antibodies to measles virus in their sera and cerebrospinal fluid as measured by the standard neutralization, hemagglutination-inhibition, and CF procedures. The development of sensitive immunoprecipitation techniques and the increased knowledge of the measles virus proteins prompted us to investigate the presence of antibodies to each of the viral proteins. This investigation led to the surprising and exciting finding [49, 55] that patients with SSPE lacked antibody to the M protein, in spite of high titers of antibodies to the other viral proteins. This finding was confirmed in other laboratories [63, 64].

Other researchers have reported a failure to detect antibodies to the measles virus M protein in the convalescent-phase sera of some individuals with uncomplicated measles or after vaccination and in the sera of some patients with multiple sclerosis [65-67]. However, it should be emphasized that such patients do not have the high titers of antibodies to other viral proteins that are found in SSPE. In addition, we have demonstrated the presence of antibodies in the sera of vaccinated individuals who were used as controls in our studies of sera from patients with SSPE [49] and in the sera of all 10 patients with multiple sclerosis examined [57]. The differences between these results probably reflect differences in the sensitivity of the techniques used; however, the important point is that in the more than 40 sera from persons with SSPE that we have examined, as well as in the sera tested by others [63, 64], there is a relative lack of antibody to the M protein in the presence of very high titers of antibodies to the other viral proteins. Furthermore, there is now evidence that suggests an explanation for the selective lack of antibodies to the M protein in SSPE.

**Evidence for lack of synthesis of M protein in the brains of patients with SSPE.** Several possible explanations for the absence of antibodies to the M protein in the presence of high levels of antibodies to the other viral proteins in the serum and cerebrospinal fluid of patients with SSPE were considered initially: (1) a lack of synthesis of the M protein in the cells of the brain; (2) rapid degradation of the protein before it became available to the immune system; and (3) a specific immunological defect in which this protein was not recognized by the immune system. We suggested at that time [49] that the first possibility appeared to be the most likely, and evidence supporting the hypothesis that the M protein was not synthesized in normal amounts in the brain cells of patients with SSPE was soon obtained; in an explant culture of brain cells that had not been cocultivated with other cells, the other measles virus proteins were synthesized, but the M protein was not [55]. In addition, some cell lines have been described that were derived by cocultivation of brain cells from SSPE patients with other cells in which the M protein is not synthesized but in which other measles virus proteins are found [64, 68]. We have now obtained direct evidence for the lack of measles virus M protein in the brains of patients with SSPE using a technique that permits immunoprecipitation and identification of viral proteins directly from brain tissue [58]. The other measles virus proteins were found in brain tissue from SSPE patients, but the M protein could not be detected. It remains to be determined whether this apparent lack of synthesis of the M protein in the brain cells is due to a defect in transcription of the messenger RNA for this protein or in the translation of the protein from the messenger RNA or whether it results from rapid degradation after synthesis.

Regardless of the precise explanation for the lack of the M protein, its absence can explain many of the virological features of SSPE. Because the M protein plays a major role in the assembly of the virus particle at the cell membrane, a lack of M protein in the cells would result in an abortive infection in which no mature infectious virus particles are produced, the internal components of the virus accumulate in the cells, and the surface glycoproteins of the virus would be exposed on the surface of the cells to stimulate high levels of antibodies. All of these findings are characteristics of SSPE.

**Brain cell restriction of virus replication as an explanation of findings in SSPE.** It should be recalled at this point that infectious virus has been recovered from the brains of patients with SSPE only after cocultivation of brain cells with cell
lines, such as HeLa or CV-1 cells, that are capable of supporting replication of measles virus [61, 62]. Once the virus is isolated, it is capable of replicating in permissive cells to yield infectious virus, which of course requires synthesis of the M protein. This sequence implies that there is a host restriction of replication of measles virus in the brain cells. The recent evidence suggests an explanation at the molecular level for such host restriction: the brain cells do not synthesize the M protein. Our current hypothesis is not that the cells of only rare individuals fail to synthesize M protein and thus these individuals contract SSPE; instead, we propose that the failure to synthesize M protein is a general property of certain types of cells in the brain. If the latter is the case, then it is not necessary to require that a mutant virus be involved in SSPE. Whether an individual develops SSPE could depend on the type and number of cells in the brain that were infected in the original episode of measles. It is pertinent that among patients with SSPE there is frequently a history of measles before the age of two years; thus, the stage of development of the central nervous system at the time of infection could play a role. A further observation that suggests a host-cell restriction on replication of measles virus in the brain which is not limited to SSPE is that in acute measles encephalitis, which though an unusual complication of measles (about one in 1,000 cases) is still much more common than SSPE, many attempts to isolate the virus had been made over the years but a successful isolation required cocultivation with other cells [69].

We have obtained additional evidence [56] that is compatible with a defect in synthesis of the M protein of measles virus in the brain in conditions other than SSPE from a patient who had measles complicated by acute encephalitis at the age of 3.5 years. Following the encephalitis the patient had persisting extensive neurological defects, developed a generalized seizure disorder which has been intractable to a variety of medications, and became severely mentally retarded. At the age of 28 years, 24 years after the episode of measles encephalitis, this patient was found to have very high levels of antibodies in her serum and cerebrospinal fluid to all of the measles virus proteins except M protein [56], a pattern similar to that seen in SSPE [49, 55]. The high antibody titers strongly suggest that the genetic information of measles virus was still being expressed in the central nervous system of this patient many years after acute encephalitis but that the M protein was not being synthesized. These findings suggest that SSPE is not the only chronic neurological syndrome related to the persistence of measles virus in the central nervous system. These results also suggest that further efforts should be devoted to searching for evidence of measles virus infection and investigating the nature of the antibody response in other patients with neurological deficits, including some individuals with mental retardation and/or seizure disorders of unknown etiology.

References

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